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VOLUME III JANUARY, 1898 No. 1

THE JOURNAL OF EXPERIMENTAL MEDICINE

FIBRINOUS EXUDATES AND FIBRINOID DEGENERATION.

BY H. R. GAYLORD, M. D.
(From the Pathological Institute of Professor Orth, University of Gottingen.)

PLATES I AND II.

No subject in pathology has been the cause of more discussion or of more radically divergent interpretation of its phenomena by scientific investigators than that of fibrinous exudates on serous surfaces.

The literature of the subject previous to 1880 shows a fairly even division of opinion. The investigators of this period form two groups, one of which believed that the fibrin was the result of exudation and coagulation, while the other attributed its presence wholly or in part to degeneration of the connective tissue forming the groundwork of the serous membrane. To mention briefly and in their chronological order the authorities who have adhered to the theory of exudation, we must begin with Cohnheim (4), who describes the classical exudation of fibrin on serous surfaces and its organization by connective tissue. Of the epithelium of the serous membrane he speaks clearly, stating that it either becomes swollen and undergoes fatty degeneration, or exceptionally through proliferation of connective tissue is dislodged, and thus robbed of nutrition, perishes.

After Cohnheim, Wagner (6) studied the question and described clearly the characters of the fibrin, which, he says, consisted of a network of fibres in which large, glistening, homogeneous clumps were nearly always present. The pleural epithelium he found in fresh, as well as older, inflammations intact below the fibrinous deposit. In closing he declares for the older view that the fibrin originates in exudation from the circulation.

Weigert (7) supports the theory of exudation, and speaks of the clumps and masses of fibrin found in the fibrinous network. Later he says (8) that the endothelium of the pleura must be destroyed to produce such an exudate, and that such a destruction of endothelium marks the boundary between tissue and fibrin.

Orth (14) accepts the theory of exudation and coagulation. The cases coming under his observation showed the usual network and the homogeneous masses. The epithelium, according to his observations, disappears early in the process, first showing proliferation of the nuclei and swelling and granulation of the protoplasm. After the disappearance of the epithelium the fibrin lies directly upon the connective tissue of the pleura.

The first to oppose the theory of exudation was Rokitansky (1). He believed that the fibrin covering serous membranes was the result of tissue "vegetation." The connective tissue first proliferated and then became a hyaline mass without structure. The new tissue obtained its nutriment partly from the vessels to be found in it, and partly from the fluid contents of the serous cavity.

Virchow (2), in his Gesammelte Abhandlungen, says: "The raw, dry, uneven fibrinous membrane is in such intimate connection with the underlying tissue that it is at times as difficult as in the case of diphtheritic membranes to determine a sharp boundary between tissue and fibrin. One merges so gradually into the other that the whole gives at once the impression that the fibrin is nothing more than degenerated tissue." Two years later he again (3) expressed himself as opposed to the theory of exudation and said that the fibrinous mass is in part nothing more than degenerated and swollen tissue.

Buhl (5), although not excluding the possibility of the fibrin having its origin in exudation, considered that the solid portions of the mass were produced by the pleural connective tissue through a process of proliferation and inflammation, and he called the product "desmoider Faserstoff." He differentiated two layers of the same, a deep and a superficial layer. The former contained blood-vessels and cells in large number, was a direct continuation of the pleural connective tissue, and found its limit always at the outermost elastic lamella. The superficial layer was free from vessels and amorphous in character, and he could occasionally demonstrate endothelial cells covering its free surface. The cases examined by Buhl were clearly, from the above description, in an advanced stage of organization.

Neumann (9), in an article on the use of picrocarmine staining in the study of inflammatory conditions, came to the conclusion that in tubercular inflammation the fibrinous masses were the result of degeneration of connective tissue. He agreed with the view of Buhl in so far as the origin of the fibrin was concerned, but considered the process a passive one and named it "fibrinoid degeneration" of connective tissue. In tuberculosis he could demonstrate the change from connective tissue to fibrin in all its stages. He found that the connective tissue first became swollen and lost its fibrillar appearance. From the red-staining connective tissue there developed shining masses which the picric acid stained yellow. The masses were often isolated in the connective tissue, but on the surface became fused together, giving the appearance of an exudation. He found epithelium on the surface but rarely, and then only in small, isolated patches. In acute pleuritis associated with croupous pneumonia and in acute purulent puerperal peritonitis he was of opinion that the deeper layers of the fibrinous mass were the result of fibrinoid degeneration. He did not, however, apply the idea of degeneration to acute cases free from connective tissue proliferation, but considered them more probably the result of exudation.

The authors quoted above, who made special investigations of the subject from 1855 to 1880, left the question but slightly changed in its general aspects. As technique improved, much of the work of older investigators who had opposed the theory of exudation was disproved, and the last quoted investigation of Neumann, which closed this first period, left the scientific world either adhering to the old theory of exudation or taking the middle stand advanced in his article.

From 1880 till 1892 the question preserved much the same status, although during this time two writers on the subject, Langhans (13), in an article on periorchitis sero-fibrinosa and pleurisy with fibrinous deposit, and Schuchardt (21), in an investigation of subacute hygroma, came to the conclusion that the fibrin was produced by a degenerative process in the connective tissue.

In 1892, through an article published by Schleiffarth (19), a pupil of Grawitz, and one year later by statements made by Grawitz himself (17), the question received a new impetus. Grawitz and Schleiffarth claim that the fibrin is the result of degeneration of connective tissue. Schleiffarth states that the tissue degenerates in one layer after another, and lays no stress on the position of the elastica. Neither of them agrees with Buhl and the last named investigators that the fibrin is produced directly from the connective tissue. Grawitz (18) advances the theory that there is an intermediate stage in which he can see cells and nuclei develop from the connective tissue and from the lymph spaces of the same, and these he calls "slumbering" cells. This idea of the development of cells from the tissue constitutes the so-called "Schlummerzellen-Theorie" of Grawitz. He believes that the cells which other observers call leucocytes are not always to be seen as such, and that the protoplasm from which they are produced can become fibrin, or that their nuclei, when formed, can remain enclosed in the fibrinous mass and form the cell elements found in fibrinous exudates. As proof of this theory, so far as its application to fibrinous exudates is concerned, Grawitz and Schleiffarth cite the regular arrangement of the cells and nuclei in the fibrinous mass, which lie parallel to the direction of the connective tissue of the serosa, and the possibility of demonstrating the epithelium or the outermost fibrous constituents of the serosa to be intact, while beneath them bands of fibrin can be found. At other times the fibrin present on the surface can be demonstrated as the result of a direct degeneration of the connective tissue.

Following the article of Schleiffarth, Apel (29), a student of Orth, published a dissertation embodying the study of twenty-eight cases of simple acute and specific inflammations of serous membranes. His conclusion was that the middle position taken by Neumann was correct. For specific cases he accepted a degeneration of connective tissue, but for acute cases he considered the fibrin the result of exudation alone. In all his acute cases (15) he found the epithelium unbroken beneath the fibrin. In specific cases he found degeneration and exudation combined. He cited a case of cancerous peritonitis on which a laparotomy had been performed during life and from which the operator had removed a large quantity of fluid which coagulated. Upon microscopic examination of specimens from this case, Apel found that beneath the fibrin covering the surface the connective tissue in many places showed the degeneration described by Neumann, and he concluded that both processes, exudation and degeneration, can occur together. As to the work of Schleiffarth and Grawitz, Apel says that in his specimens he was unable to find any appearances which could possibly suggest the development of cells and nuclei from the intercellular substance of connective tissue.

In a review of Grawitz's atlas Marchand (22) says: "One of the most remarkable acquisitions to modern science, which makes its appearance in this atlas, is that fibrin found on the surface of serous membranes is not an exudate, but degenerated intercellular substance. The fibrinoid pseudo-membrane does not rest upon the pleura, but is the result of degeneration of the superficial layers of the same, in fact (in the intestine) this degeneration can extend through the entire thickness of the muscularis. In short, the process is one of direct degeneration of connective tissue; otherwise how were it possible to account for the Schlummerzellen (alias leucocytes) found in the fibrin if they were not produced where they are found?" In support of his statement regarding fibrin, Grawitz gives photographs (Plate XXIX of his Atlas) in which Marchand says he can see the partly preserved epithelium underlying the mass. In closing Marchand says: "It is well known that in certain cases fibrin can be produced by exudation in the tissue itself: why not in the serosa?" "No one who had not a theory to support would confuse this condition with a pseudo-membrane, or on careful examination (without special staining methods) would fail to find the connective tissue fibres in the fibrin."

In answer to this criticism of Marchand, Neumann again entered the discussion in support of the theory of fibrinoid degeneration as applied by Grawitz and Schleiffarth, but he attempted to separate that phase of the question from the theory of slumbering cells, stating that the discussion involved "two questions, where the fibrin had its origin and whence came the cells found in it." Neumann now extends his theory, applied originally to chronic processes, to acute as well. This new position is based largely upon a case of acute pleurisy with fibrinous deposit, covering which he found a continuous layer of epithelium which he concludes is the pleural epithelium in its original anatomical position, and therefore that all fibrin below it is derived from degenerated connective tissue. He considers as characteristic of this degeneration the hyaline masses and bands found in the deeper layers of pseudo-membranes. These bands are, according to his investigations, nothing more than connective tissue fibres in partial degeneration, and represent a transitional state between the connective tissue below and the completely degenerated mass (fibrin) above.

In answer to this article of Neumann there appeared an exhaustive argument against it by Marchand (23), and an address on the subject before the pathological section of the Naturforscherversammlung in Frankfurt by Professor Orth, based upon the specimens of Vassmer and myself.

In reply, Neumann (12) published a short restatement of his position, laying special stress upon the points which he did not consider to have been satisfactorily met in Marchand's article. It is especially with these last two publications of Neumann (11 and 12) that the present article proposes to deal.

Neumann (12) says: "As fibrinoid degeneration of connective tissue, I have described a condition, in my experience widely distributed, in which the connective tissue degenerates, forming a mass which resembles macroscopically and microscopically, and gives the same chemical reactions as, fibrin the result of coagulation of blood. This change is accomplished by the bundle of connective tissue swelling, and melting together in such a manner that fibres can no longer be recognized as such, the whole forming a highly refractive mass, in which can be seen at first the nuclei of connective tissue cells." Morphologically the change can be compared to amyloid degeneration in which the tissue undergoes a change forming amyloid substance, except that in the fibrinoid metamorphosis the connective tissue undergoes a degeneration the result of which is a mass identical with blood fibrin. The best examples of this degeneration, he says, are to be found in the study of inflamed serous membranes. In particular he cites that form of inflammation found in the pleura overlying portions of hepatized lung, and states that the mass of fibrin found in such cases is the result of such degeneration for the following reasons: first, he finds that the pleural epithelium covers the mass; second, a sharp boundary between the fibrin and the underlying connective tissue at the beginning of the process does not exist. The epithelium which may be found under the fibrin he agrees with Schleiffarth in explaining as endothelium of a lymph channel, which appears only after the process is well advanced and marks the boundary between active process and degenerated mass.

To this can be added a case of chronic fibrous periorchitis reported by Sultan (27), in which the mass of fibrin covering the tunica vaginalis lay between two continuous layers of epithelium, one covering the mass and the other at the point of demarcation between fibrin and tunica vaginalis. This case he explains by assuming that the epithelium covering the mass is the original epithelium of the tunica vaginalis which with the mass has been thrown off, while the layer of epithelium underlying the mass he believes to be the result of regeneration.

The point made by Neumann that at the beginning of exudative processes in serous membranes no cell demarcation exists, does not agree with my experience. A careful examination of all cases coming to hand in the Pathological Institute during the last ten months shows overwhelming evidence to the contrary. The fresher the condition the more perfectly preserved has been the epithelium, a statement which applies to simple and septic cases of pleurisy and pericarditis. Figs. 1, 2 and 3 (Plate I) are photo-micrographs of such cases and can leave no doubt as to the character of the cells. That such a cell demarcation is not referable to a collection of endothelial cells in a lymph space seems to me to be conclusively proven by the following facts: The mass of fibrin lies directly upon the cells; there is no connective tissue between the row of cells and the fibrin, and in Fig. 1 (Plate I) the fibrin is separated from the cells which are clearly attached at their base. Moreover the cells appear continuous in all sections cut from the same block as well as from blocks taken from different parts of the pleura or pericardium, which could not happen if we were dealing with cells arranged in lymph spaces, in which case we would find intervening tissue. That these cells, as Neumann says, do not make their appearance until after the process is well advanced, and that they are necessarily associated with a cellular infiltration in the connective tissue of the serosa, is opposed by the appearances in Fig. 3 (Plate I) in which the exudate is very fresh and the connective tissue free from cellular infiltration. Vasmer (16), who made an exhaustive study, by means of orcein staining, of the relation of the elastica of the normal pleura and pericardium to the epithelial covering, found that the epithelium when not arranged directly over the elastica is separated from it by only a small layer of connective tissue, which in the pericardium is less than in the pleura.

From these facts it is safe to take the stand that where we find no change under the elastica it is very improbable that the small amount of connective tissue lying over it can have furnished the entirely disproportionate amount of fibrin so commonly observed. If, moreover, we find connective tissue between the elastica and the fibrinous mass, it seems evident that we require no cell demarcation to show that the fibrin has not been produced at the expense of the underlying tissue.

Neumann recognizes the relation of the fibrin to the outermost elastic lamella of the pleura and pericardium, and states that the process, *i. e.* fibrinoid degeneration, finds its limit at the elastica. Buhl also accepted the elastica as the boundary of the process. Schleiffarth, on the contrary, pays no attention to this well-known anatomical landmark, and simply says that the fibrin is the result of the degeneration of the connective tissue which extends inwards, one layer after another becoming degenerated.

In all cases where I was not able to demonstrate epithelium this relation of the elastica to the fibrinous mass has invariably shown the fibrin lying over the elastica, and in the majority of cases a distinct amount of connective tissue between the elastica and fibrin. In Figs. 1, 2, 3 and 4 (Plate I) the elastica is stained with orcein and shows clearly this relation. I cannot speak too strongly of the value of this method, as in many cases at first doubtful the staining of the elastica proved the only means of clearly determining the relation of the exudate to the underlying structures.

Neumann describes as characteristic of simple non-septic cases a fine fibrinous membrane, which is found at the very beginning of the process. Such membranes, he says, cannot be removed with the forceps without tearing away portions of the underlying tissue. These membranes he considers to be the very beginning of the process, before the fibrin has been thrown off and while it is still connected to the tissue, from the degeneration of which he supposes it to originate. It is in these cases that he finds no epithelium under the fibrin and states that a sharp line of demarcation between fibrin and connective tissue does not exist. Marchand (22), speaking on this point, says: " Every beginner knows that it is exactly the freshest fibrinous membranes which are the easiest to remove." My experience agrees with this statement of Marchand, except that the very fresh exudates, owing to their extreme thinness, could not be removed in large pieces. It was always in such cases that I found continuous epithelium under the mass. The case described by Neumann, in which the epithelium covered the fibrin, certainly did not present the condition usually found. The only other observer who distinctly describes the condition noted by Neumann is Buhl. He observed, in cases which we now know to have shown an advanced state of organization, small patches of epithelium covering the granulations.

Neumann says he has in a number of fresh cases found epithelium covering the exudate. His method for observing the same is as follows: The fibrin carefully removed from the fresh preparation is spread with its outer side upward on a glass slide, and then covered with salt solution. The specimen can be examined at once, or he recommends staining it on the slide with acetic acid vesuvin, which gives the nuclei a deep brown, the fibrin a yellow color. All specimens coming to the Institute have been examined by this method, and, while I have here and there found cells such as Neumann describes, I have never failed to find on the under surface large patches of undoubted epithelium, and in many cases have been able to demonstrate in the hardened section continuous epithelium under the fibrin. I should consider the evidence obtained by this method exactly the opposite to that which Neumann claims for it.

In November, 1895, seven months before the appearance of Neumann's first article, I undertook, at the suggestion of Professor Orth, a series of experiments on animals, to ascertain if fibrin obtained from blood, when introduced into the serous cavities, would organize as such, and what microscopical appearances such specimens would present as compared with exudates in the human body. These experiments showed that fibrin, introduced into serous cavities in animals under aseptic conditions, will organize in the usual way in which fibrinous exudates organize, and presents at every stage of the organization the same processes which we find in the human body.

The scope of the experiments covered, first, the introduction of fibrin as such; and, second, the injection of coagulable fluid which produced the fibrin in the serous cavity. As fibrin, I introduced that obtained from whipping blood (slightly hardened in formalin) and coagulated blood plasma. As coagulable fluid, I injected plasma obtained from horses' blood, first with the addition of a fibrin ferment, and then alone.

Of the experiments, eighteen were successful and gave identical results. The results for the pleural and peritoneal cavities were the same, but the difficulty of finding the fibrin after introduction into the peritoneal cavity led me in my later experiments to use the pleural cavity alone. The first five experiments were made with fibrin obtained by whipping, and were so planned as to show the organization at different stages.

Experiment I.—Grey rabbit. Introduced into right pleural cavity two pieces of fibrin, size of small pea.

Autopsy seven days after operation. Incision shows no signs o tion. Pleura shiny. No fluid in pleural cavity. Fibrinous m changed in appearance is found adherent to lower lobe of right its outer inferior border. It appears to lie in an impression in the of the lung, and its base is surrounded by a secondary fibrinous e Surrounding pleura shows no change.

Lower lobe of lung and fibrin hardened in Müller-formalin s Embedded in paraffine. Microscopic appearances. Section shows mass of fibrin adherent at its base to the parietal pleura. Pleural lium intact and shows evidences of proliferation at several places lying the fibrin. Original mass of fibrin is surrounded by a se fibrinous exudate containing leucocytes, which also penetrate i fibrinous mass proper. Lung tissue directly underlying the fibri atelectasis. Fibrin, both that introduced and that secondarily i give the usual color stain with Weigert's method, and the yellow r with Neumann's modification of the picrocarmine stain.

Experiment II.—White rabbit. Introduction into right pleura of two pieces of fibrin stained with aniline-water gentian-violet. experiment was made to ascertain earliest appearance of seconda date, hence the staining of introduced fibrin before introduction.)

Autopsy forty-eight hours after introduction. Both pieces o found adherent to costal pleura covering cartilages. Pleura s red zone 1½ mm. wide, surrounding base of fibrinous masses.

Costal cartilages, to which fibrin is adherent, dissected out a adherent mass hardened in Müller-formalin. Embedded in p Microscopic appearances (picrocarmine). Fibrinous mass stain surrounded by secondary fibrinous exudate stained yellow. A fe cytes can be seen in the exudate. Pleural epithelium beneat intact. At the sides of the preparation we have the pleural epi covered by the secondary exudate alone, giving the picture of exudate. Blood-vessels of the pleura distended with blood.

Experiment III.—Grey rabbit. Introduced into right pleural two pieces of fibrin size of small peas.

Autopsy eight days after operation. Both pieces of fibrin organized, being attached to costal pleura. Pleura at base of appears normal. Both pieces removed with intercostal tissue a dened in Müller-formalin solution.

Microscopic examination. Entire fibrinous mass infiltrated wit cytes. At base the mass is the seat of proliferation of connective which has organized the lower third of same. Fibrin of homo character, except at base where we find glistening masses, many o are isolated in the newly proliferated connective tissue. Fine blood-vessels can be traced running from the superficial vessels of the pleura into the newly proliferated tissue. Pleural epithelium intact in patches underlying the mass, but cannot be demonstrated where we find the proliferation of connective tissue. Examination of the free surface of the mass shows it to be covered with a single continuous layer of cells resembling epithelium. This arrangement of cells when traced to the sides of the fibrinous mass shows them to be a direct continuation of the pleural epithelium (Plate II, Fig. 10). At the highest point of the mass the epithelial layer is flatter that at the sides, where the character of the pleural epithelium is preserved. In the crevices of the mass we find that this proliferation of the cells has produced several layers, but on the free surface the arrangement is that of a simple continuous layer. At one side of the preparation the proliferation is most remarkable. Projecting from the main mass of fibrin is a small band which touches the adjoining pleura with its extremity, leaving between its under-surface and the pleura a small free space. At the point of contact of this band with the pleura the epithelium has proliferated upon the under as well as the upper surface of the fibrin, so that counting from below outwards we have three layers of epithelium: first, pleural epithelium proper; second, a row of epithelium covering the under surface of the mass and continuous with the pleural epithelium of the same side; third, covering the outer surface a layer of cells continuous with the pleural epithelial layer extending beyond the point of contact. At the outer corner the fibrin has not fallen in direct contact with the pleura and we find the cells proliferated one over another for several layers perpendicular to the pleura until it reaches the fibrin, where it becomes a simple layer spreading out over the mass. Beneath the fibrin are several crevices left by irregularities in the mass. In these the pleural epithelium which remains intact has proliferated in such a way as to line them with a continuous layer of cells. Between these crevices, where the fibrin has come in direct contact with the pleura, no epithelium can be demonstrated and we find the connective tissue of the pleura widely proliferated into the fibrin. In one of the above described crevices we find the preserved pleural epithelium broken through by a single band of proliferating connective tissue extending into the fibrinous mass above, which, as in the other crevices, is covered with a single layer of epithelium (Plate II, Fig. 11). The elastica is continuous beneath the mass and we find no changes in the tissues underlying it.

Experiment IV.—Black rabbit. Introduced two pieces of fibrin into right pleural cavity.

Autopsy fourteen days after operation. Both pieces of fibrin oı to costal pleura, which with ribs are dissected out in one piece. cified. Hardened in Müller-formalin. Microscopic examinatic find the fibrinous mass to be invaded by proliferated connectiv which has entirely organized the lower two-thirds. At the sid main mass is to be seen an isolated glistening fibrinous clum II, Fig. 8). This mass, stained with picrocarmine, shows a l fractive index and brilliant yellow color. Its appearance is d hyaline, and at its edges we find that the connective tissue has br way into the mass. No pleural epithelium can be seen bene fibrin. Blood-vessels can be demonstrated in the organized Entire mass, as in Experiment III, covered with a layer of epi continuous with the pleural epithelium at both sides (Plate II,

Experiment V.—Grey rabbit. Introduction of fibrin as in experiments.

Autopsy four weeks after operation. On costal pleura we fin somewhat smaller than the piece of fibrin introduced, and more in appearance. Dissected out. Hardened in Müller-formalin.

Microscopic appearances. Mass largely converted into conne sue. At base are to be seen here and there small bands of fibri appear hyaline in character. As we approach the surface thes are more frequent and larger until we find only fine bands nective tissue growing into the fibrinous mass proper. The ent covered with epithelium as in previous experiments. Elastic Connective tissue beneath elastica unchanged.

Experiment VI.—The fibrin used in this experiment was pro the action of a fibrin ferment on plasma obtained from horse The ferment was made after the method of Alexander Schn Rauschenbach by expressing the contents of a fresh lymph gl diluting it with water. By this method the plasma placed in crystal formed in two minutes after the addition of the fermen gelatinous clot. Microscopic examination of this clot showed homogeneous in character. Careful examination of several s failed to show any hyaline-like masses.

Operation. Introduced into right pleural cavity of grey rabb fibrin immediately after coagulation.

Autopsy seven days after operation. Fibrin found organized pleura near point of incision. Dissected out, hardened, emb paraffine.

Microscopic examination. At base of section part of interco cle. Between muscle and fibrin connective tissue, which in dee runs parallel to muscle; in outermost portion it consists clearly of newly proliferated structure which penetrates into the fibrin. Isolated hyaline masses, giving reaction of fibrin with all staining methods, are to be found in the newly proliferated tissue. These masses are to be seen only where the organization is advanced. The fibrinous mass proper presents in part the homogeneous character described, and in part a fine network of fibres. Elastica intact except where broken through by incision, where we find newly proliferated tissue. No hyaline masses or fibrin found beneath elastica. The whole fibrinous mass covered by a *single* epithelial layer continuous with the pleural epithelium.

Experiment VII.—Grey rabbit. Small incision made between ribs on right side opening pleural cavity. Simultaneous injection through this incision of plasma and ferment, prepared as in previous experiment.

Autopsy in eight days. Fibrin organized to costal pleura. Hardened and embedded as usual.

Microscopic examination. Fibrin presents same appearance as in previous experiment. At base, organization surrounding hyaline-like masses. Through the entire mass we find the lymph cells from the ferment enclosed in the fibrin. In many places these cells and nuclei follow one direction, at the surface parallel to it, and in deep parts pressed into rows. Entire mass covered by a continuous layer of epithelium, beneath which are several layers of connective tissue. This organization of the fibrin on the surface can be traced from the sides where the proliferating tissue forms a continuation of the organization at the base. We find the connective tissue, in this zone beneath the epithelium, following a direction parallel to the surface, while the nuclei and leucocytes in the adjacent fibrin have the same parallel distribution. Plate II, Fig. 12, taken from this specimen, shows the epithelial covering, the organization beneath it, the leucocytes in the fibrinous mass, and the proliferating connective tissue extending upward from the base. At base of mass can be seen the remains of the incision where the elastica is broken. In sections where the incision does not appear the elastica is intact, with tissue below it free from fibrin and hyaline masses.

Experiment VIII.—Injection of 20 cc. of plasma into right pleural cavity of grey rabbit.

Autopsy one week later. Upon opening pleural cavity a flattened fibrinous mass, 18 by 12 mm. in surface dimensions, and 3 mm. in greatest thickness, is found. The same appears to be unattached. Hardened, embedded and cut. Microscopic examination of this fibrinous mass shows the same to be covered by a single layer of epithelium, except at one point where the epithelium fails and the mass is the seat of organization. This area forms about one-twentieth of the cir ence and the organization extends into the mass for but one-fifte its diameter. Fibrin homogeneous.

Further experiments merely confirmed the results already and do not require special mention.

Summing up the results of these experiments, we find the fo characteristics in all: Fibrin introduced as such, and fibrin-f fluids when injected into serous cavities, become adherent serous membrane. The pleural epithelium remains intact un fibrin until the process of organization begins. The epitheliun pleura has the power to proliferate sufficiently to cover larg (Experiment VIII). This extensive proliferation was found experiments after the sixth day. In one case (Experiment which the autopsy was made forty-eight hours after introducti proliferation had not begun.

The proliferation of epithelium occurs before the organiza connective tissue. It is possible to find the epithelium pr below the fibrin and its surface covered by an epithelial la result of proliferation from the sides (Experiment III).

After the proliferation of epithelium over the mass, t nective tissue follows the same direction from the sides, for layer of connective tissue organization on the surface bene epithelium. The principal organization takes place at the bas mass. This organization by connective tissue tends to separat from the main fibrinous deposit. These masses appear hy character. They are often found lying parallel to the co tissue fibres. Where cells or nuclei are injected with a fibrin- fluid, they become enclosed in the fibrinous mass and by cess of coagulation are often arranged in rows.

An application of these facts to the statements made by N permits the following deductions: Neumann says that the epi covering the fibrinous exudate in his case was the pleural epi in its original anatomical position, and the fibrin was the r degeneration of connective tissue lying beneath it. He excl possibility of the fibrin being produced by exudation beneath the epithelium. That the epithelium could have been derived from and merely adherent to the opposite pleural layer he considers to be excluded by the presence of a pleural exudate. The explanation of Marchand that the epithelium was the result of proliferation (23) he sets aside with the statement that " in acute cases such a proliferation is inconceivable " (12).

In answer to Neumann's statement, and in support of Marchand's explanation, my experiments show an unfailing proliferation of epithelium on the surface in all cases in which the fibrin remained seven days in the serous cavity. The proliferation of epithelium in chronic cases, to which the observations of Buhl quoted by Neumann belong, has been observed by Riese (28), who found in tuberculous synovial cavities masses of fibrin covered by a layer of " endothelium " continuous with the " endothelial " layer of the sac. He distinctly states that he believes the cells to have proliferated over the mass. This observation agrees with those of Michon (26), who observed the same condition in synovial cavities. These cases are quoted by Neumann as examples of degeneration producing fibrin beneath an epithelial covering in its original anatomical relations.

Of still greater interest, in the light of these experiments, is the case quoted by Neumann (11), reported by Sultan (27), in which the fibrin lay between two layers of epithelium. Neumann believes that the superficial epithelial layer is the original epithelium, and that the epithelium marking the boundary between fibrin and connective tissue has been produced by regeneration. This case is, however, clearly one in which the original epithelium remained partly intact and the epithelium from the sides proliferated over the mass (compare Experiment III).

From these cases, experimental and others, we may safely conclude that the presence of epithelium overlying a fibrinous mass does not constitute proof that the fibrin was produced beneath it.

That the hyaline masses described by Neumann as characteristic of fibrinoid degeneration are in no way characteristic of such a process is shown by the presence of the same in all my experiments where

organization was advanced and where the process in no case e
beneath the elastica. In further proof of the same, I have
possession a specimen of fresh thrombosis of the aorta in whic
middle of the thrombus mass, where no question of degener
connective tissue can be entertained, are to be seen hyalin
and clumps easily distinguishable from the surrounding fib

Finally, the arrangement of cells and nuclei in rows thro
fibrin mass is not suggestive, as Neumann, Grawitz and Sch
would have it, that the same are remaining connective tissue nu
owe their arrangement to this fact.

The older theory that the cells found in fibrinous exud
leucocytes enclosed in the fibrin is in no way impaired by th
vation of Neumann, Grawitz and Schleiffarth. On the contr
arrangement tends to show that the cells were present when t
was in a fluid condition and that they owe their arrangemen
process of coagulation.

TUBERCULOSIS.

The microscopic appearances found in the fine, closely
fibrinous masses on the pleura covering the affected portions c
culous lungs are so different from those which characterize t
just described that they may well be considered separately.

Here we must at once allow that the characteristic ap
described by Neumann does to a large extent suggest a dege
of the connective tissue and the production directly from the
fibrin. So intimately adherent are the fine bands of fibri
connective tissue, and so diversely distributed between its fib
we are led at once to the belief that here we are dealing with
ent process. It is only when we return to the elastica that t
sary evidence it wanting.

It was the lack of this evidence which led Professor Ort
address at Frankfort, based on the specimens of Vassmer and
to state that " no definite position can be taken on this questi
the fibrinous masses can be demonstrated under the e
Ziegler (30) describes the condition found in such cases as
degeneration of connective tissue similar to amyloid degenera

does not agree with Neumann's explanation of the same. He regards the change as one of degeneration.

As this investigation has been concerned principally with that portion of the question dealing with acute non-specific processes, my experience with the tuberculous cases has not been sufficient to base an opinion on negative evidence. In no case have I been able to demonstrate the masses beneath the elastica. So different are the microscopic appearances in these cases from those found in the acute processes already presented that I add for comparison three photo-micrographs from a case of tuberculous pleurisy. We see here the masses and bands described by Neumann, which are by their distribution suggestive of a direct degeneration of connective tissue into fibrin.

Plate I, Fig. 5, shows a section through the pleura stained by Weigert's method. We see the masses of fibrin covering the surface and extending into the connective tissue. The connective tissue is infiltrated with cells and shows cross sections of blood-vessels.

Plate I, Fig. 6, shows a section of the same pleura with the elastica stained. Here we see that the masses lie above the elastica, and that in fact the amount of connective tissue described by Vassmer as normal lies undegenerated above the elastic stripe. Therefore all connective tissue above this undegenerated zone must at one time have been produced by proliferation, and if the fibrin is the result of degeneration, it is degeneration of a new tissue alone and not of the normal structures of the pleura.

Plate II, Fig. 7, shows the masses at the surface of the pleura and the oval nuclei described by Neumann, which he believes to be the free nuclei of the degenerated connective tissue.

Such cases can be explained, as Marchand (23) explains them, as merely the irregular organization of a previous exudate which isolates masses of fibrin.

The parallel direction of the bands of fibrin and their relation to the connective tissue is not suggestive of the picture seen in the organization of exudates, in which the young connective tissue often grows perpendicular to the pleura and seldom shows such a regular arrangement of its fibres. We need only the confirming fibrin beneath the elastica to solve the problem and separate these cases from the exudative form of pleurisy to which they bear but a very resemblance. Until the supporters of the theory of fibrinoid deg tion can establish this fact the question of the true character of culous cases will remain in doubt.

In closing I wish to take the opportunity to express my tha Professor Orth for the material which he has placed at my di and for the kind advice and support he has given me at all during this investigation.

DESCRIPTION OF PLATES I AND II.

PLATE I.

All of the figures are photo-micrographs. Objectives of the Winkel- system were used.

Fig. 1, × 150. Objective, 4.5 mm. Ocular No. 2. Stain, Van Gieson, Section of pleura covering consolidated lung from case of pneumoni

At *a*—*a'*, pleural epithelium intact. Here and there single, detach thelial cells adherent to fibrin *c*. At *B*, elastica stained with orce tween *a* and *B*, hæmorrhagic infiltration beneath epithelium.

Fig. 2, × 110. Obj. 4 mm. Oc. No. 1. Stain, hæmalum, orcein. from case of purulent pericarditis.

At *c*, thick fibrinous exudate containing leucocytes. *a*—*a'*, con pericardial epithelium. *B*, elastica stained with orcein. Between *a* layer of healthy connective tissue. *D*, heart muscle, cross section.

Fig. 3, × 110. Obj. 4 mm. Oc. No. 1. Stain, hæmalum, orcein. of pleura covering hæmorrhagic infarct of lung.

c—*c'*, fibrinous exudate containing leucocytes. *a'*—*a'*, continuous epithelium. *B*, elastica stained with orcein. Between *a'* and *B*, l healthy connective tissue. *D*, hæmorrhagic infarct of lung.

Fig. 4, × 110. Obj. 4 mm. Oc. No. 1. Stain, picrocarmine, orcei tion of adherent pleural surface, between adjacent lobes of lung fr of pleurisy.

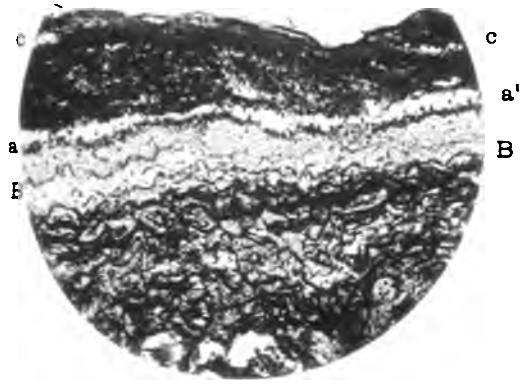
c—*c'*, point at which adjacent surfaces are adherent and in which seen fibrin mass at *a*. *A*—*A'*, remains of pleural epithelium of upper At *a'*, cells of lower surface below fibrin mass. At *B* and *B'*, elastica with orcein. Between *B* and *c*, healthy connective tissue.

Fig. 5, × 60. Obj. 8 mm. Oc. No. 1. Stain, hæmalum, Weigert fibri Section of pleural surface covering tuberculous lung.

A, fine fibrinous bands running parallel with the surface. *c*, fi blood-vessel. *f* and *f'*, fibrinous exudate in alveoli of lung.

Fig. 6, × 110. Obj. 4 mm. Oc. No. 1. Section of pleura from sa as Fig. 5. Stain, Van Gieson, orcein.

a, fibrinous mass covering the surface; *a'*, isolated bands of fib elastica stained with orcein. *c*, connective tissue above elastica. layer of serosa.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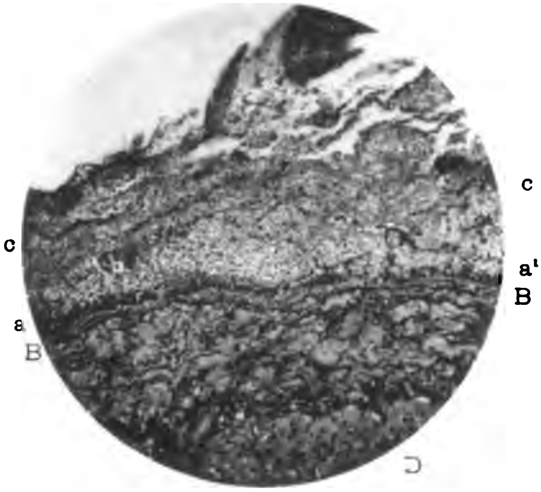
1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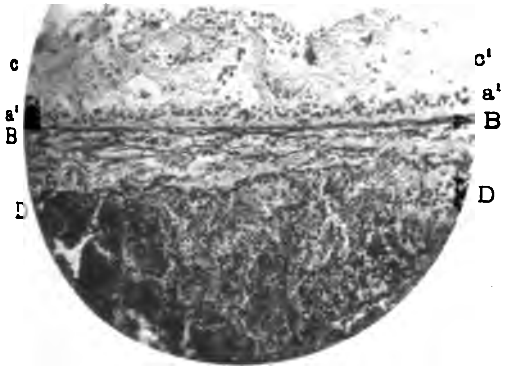
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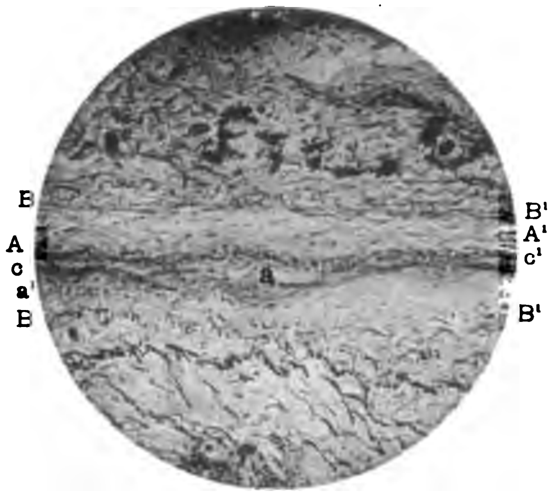
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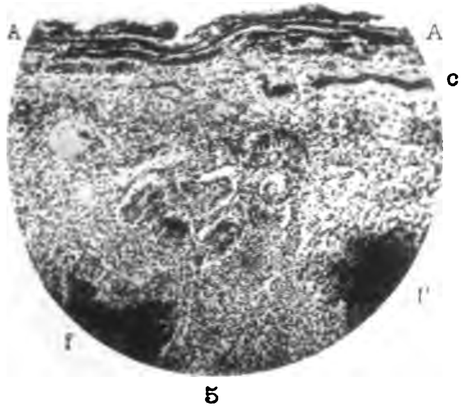
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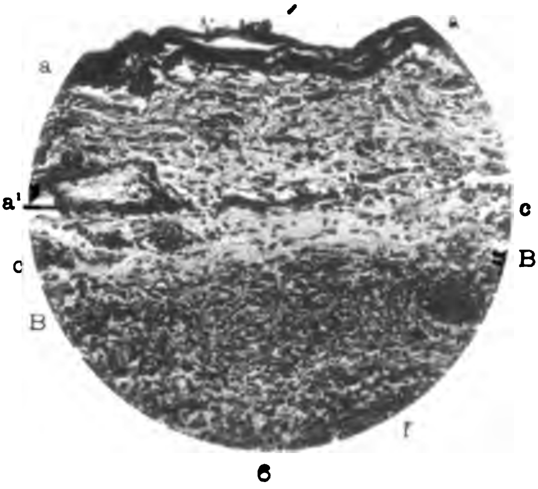
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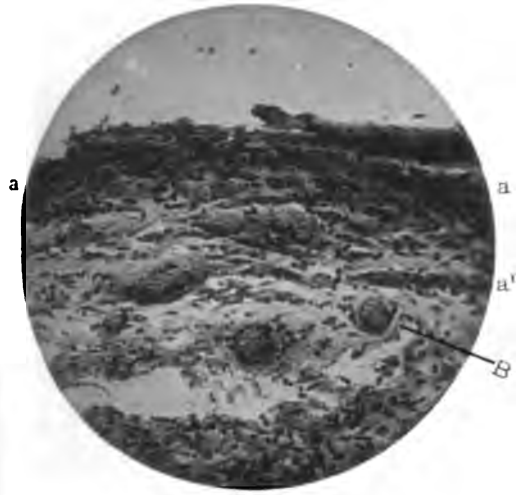

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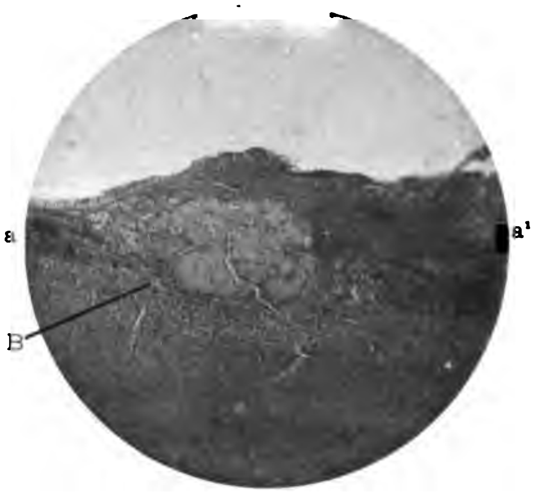

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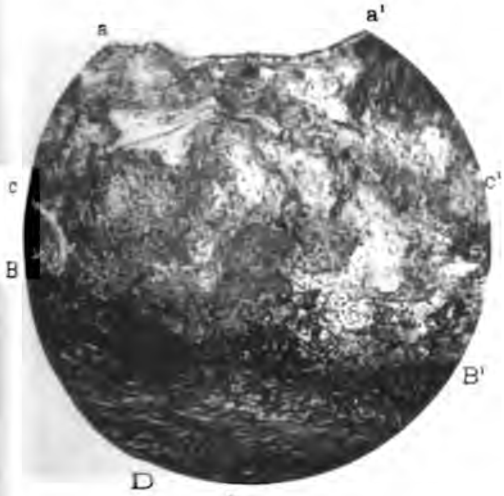

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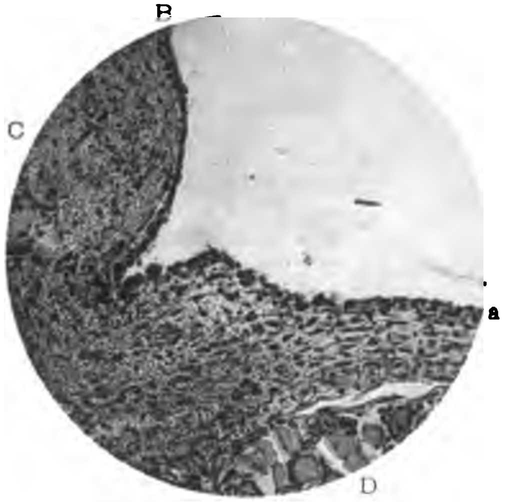

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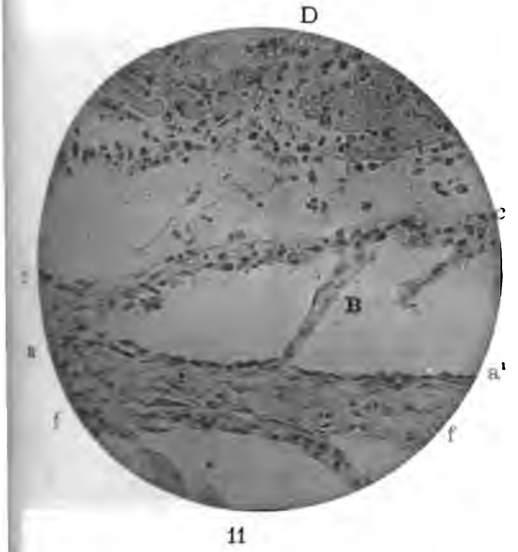

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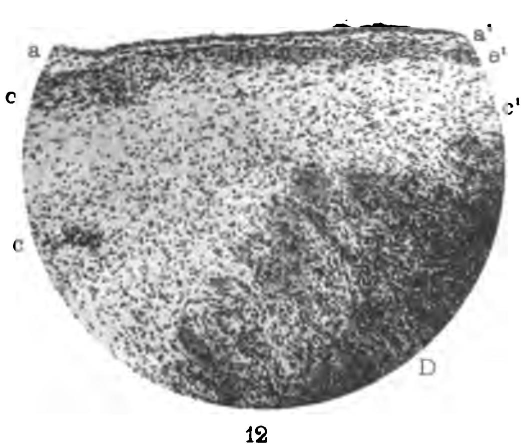

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PLATE II.

Fig. 7, × 110. Obj. 4 mm. Oc. 1. Section through pleura. Same case as in Figs. 5 and 6.

a, fibrinous masses covering surface of pleura. *a'*, single fibrinous band separated from mass by connective tissue. Fibrinous mass and surrounding tissue infiltrated with cells, many of which have oval nuclei. At *B*, distended blood-vessels.

Fig. 8, × 30. Obj. 16 mm. Oc. 1. Stain, picrocarmine.

Section of fibrin in organization from Experiment IV. *a*—*a'*, connective tissue surrounding glistening hyaline clump at *B*.

Fig. 9, × 60. Obj. 9 mm. Oc. 1. Stain, hæmalum. Section of fibrin in organization from Experiment IV.

a—*a'*, epithelium covering fibrin. *c*—*c'*, fibrin consisting of hyaline masses and clumps. *B*—*B'*, organization at base of fibrin. *D*, connective tissue of serosa.

Fig. 10, × 110. Obj. 4 mm. Oc. 1. Stain, hæmalum. Section of organized fibrin from pleural cavity of rabbit. Experiment III.

C, fibrin organized to pleura. *a*, pleural epithelium continuous with layer of epithelium *B*, covering fibrin. *D*, intercostal muscle.

Fig. 11, × 160. Obj. 3 mm. Oc. 1. Stain, alumcarmine. Section at base of organized fibrin from pleural cavity of rabbit. Experiment III.

a—*a'*, pleural epithelium broken through by band of connective tissue *B*. *c*, proliferated epithelial cells on under surface of fibrin *D*. Between *a'* and *c*, crevice beneath mass. *f*, connective tissue of pleura.

Fig. 12, × 70. Obj. 7.5 mm. Oc. 1. Stain, hæmalum. Section of fibrin mass from Experiment VII.

a—*a'*, single continuous layer of epithelium covering fibrin mass *c*—*c'*. *D*, connective tissue organizing fibrinous mass from beneath. *e*—*e'*, connective tissue organizing mass from surface.

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THE FATE OF THE GIANT CELLS IN HEALING TUBERCULOUS TISSUE, AS OBSERVED IN A CASE OF HEALING TUBERCULOUS MENINGITIS.

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PLATE III.

The investigations into the nature and the importance of the giant cells of tuberculous tissue have not led to uniform results. The views differ more particularly with respect to the genesis and to the vitality of these peculiar elements.

While Rokitansky,* Virchow and others had observed, and in part described, the giant cell, it remained for Langhans, in 1868, to first give a full and satisfactory account of these cells, and to advance the two most prominent hypotheses in regard to their origin; namely, that they are the product of one cell, or formed by the fusion of many cells. The investigations of Langhans were soon followed by a very large number of studies concerning the giant cells of tuberculous and of other inflammatory, as well as of resorptive, processes. The literature upon the mode of formation and the nature of giant cells is very extensive, and it would not materially advance the object of this article to review it completely. I would, however, refer to the studies of Schüppel, Köster, Heidenhain, Weiss, Ziegler, Brodowsky, Marchand, Ranvier, Baumgarten, Cornil, Malassez, Martin, Arnold, Kiener, Weigert, Metchnikoff and his students, Taber, Kostenich and Wolkow, Krückmann, Welcker, and of many others, as indicating the amount of work bestowed upon the problems connected with multinucleated giant cells. The majority of these investigators review the .pre-existing literature more or less thoroughly. Thus Marchand, in 1883, in his article on the giant cells that occur in connection with the absorption of foreign bodies, refers to no less than forty-five previous publications bearing on the subject of giant cells in general.

Koch's discovery of the tubercle bacillus, and the further demonstration of the important relations of this bacillus to the giant cell, g to continued vigorous investigation and speculation concerni Langhans cell. While the views of the authors in regard to the of the giant cell of tuberculosis are so divergent and the possit different modes of development so marked as to indicate that likelihood, the giant cells may indeed be the outcome of more tl process, the opinions concerning the vitality and the importance element have differed in a more precise and distinct manner, so 1 investigators may be separated into two quite well defined namely, those who, headed by Baumgarten and Weigert, regard as an element on the way to destruction, as necrobiotic from inception; and, on the other hand, those who, led by Metchnil convinced that the giant cells of tuberculosis are living, active fensive (mesodermal) cells.

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* The references to the literature are arranged alphabetically at the end of the article.

Baumgarten, in his classical study of the histology of tuberc cludes that the giant cell is the product of multipolar nuclear in a single mesodermal or epithelial cell, the body of which divide on account of degeneration in its interior, due to the nec action of the bacilli that it usually harbors. Weigert agrees witl garten and has vigorously opposed the contradictory claims of M koff and his followers.

In his original communication concerning the bacillus of tube Koch is inclined to regard the giant cells as somewhat permar ments possessing more vitality than the bacilli they usually cont holds that the bacilli are able to persist in the giant cell simply one generation succeeds another. He also points out that degenera lary forms are not infrequently met with in the interior of gia Later Koch seems to favor more directly Weigert's opinion. I koff has persistently championed the view that the giant cells ar sive, mesodermal phagocytes, produced in various ways, at times l nuclear division, at times by indirect segmentation of the nucl still at other times by the fusion of epithelioid mesodermal cells special nuclear changes. In one of his first articles on this su details the histological changes produced by the bacillus of avia culosis when injected into the small rodent, Spermophilus gut which our gopher is related. He shows how the bacilli are take the numerous giant cells that come into existence, and in the in which they undergo marked changes, both as to staining and which must be regarded as clearly retrogressive in character. He more demonstrates that giant cells may multiply by simple fissi out mitosis, and also describes appearances that indicate that more giant cells may coalesce into a larger cell complexus.

Ssudakewitsch brought further proof of the activity of giant cells by showing that the giant cells found in the skin in an Eastern disease, pascha-churda, and in lupus, are capable of digesting elastic fibres.

Arnold, to whom we owe extended studies upon the manner of formation of, and the nuclear changes in, giant cells, was at one time inclined to divide giant cells into progressive and retrogressive forms, and to designate those of tumors as belonging to the former, and those of tuberculous tissue to the latter class; but after he had been able to demonstrate that even the tuberculous giant cells have the power of further development he no longer regarded any such distinction as justifiable. The fact that giant cells are destroyed in tuberculous tissue does not in any way invalidate this statement, because this fate they suffer in common with all the cells of this tissue—the point being that the giant cells do not have in them the seed of retrogressive metamorphosis from their very origin.

Welcker, not long ago, repeated the original experiments of Metchnikoff with avian tuberculosis upon Spermophilus guttatus. He did not find any evidences of multiple karyokinesis in the epithelioid cells and questions this mode of formation for giant cells. He regards direct nuclear division as the most frequent mode of formation, but does not exclude fusion. He could not demonstrate the amœboid movement of the giant cells which was claimed for them by Metchnikoff. He found in the interior of the giant cells peculiar sausage-shaped masses that he demonstrated to contain iron, and which he regards as bacilli changed by cellular activity.

The concentric, lamellated, calcareous concretions, described by Schüppel in tuberculous lymph nodes, and also briefly referred to by Ziegler in the seventh edition of his work on Pathological Anatomy (page 97), are regarded by Metchnikoff as due to the calcification of tubercle bacilli in the interior of cells. According to Metchnikoff the process of calcification can be well traced in the Algerian rat, an animal relatively insusceptible to tuberculosis, in which the tuberculous tissue, even many months after the experimental inoculation, consists of living epithelioid and giant cells, the latter enclosing dead bacilli in the form of the characteristic earthy bodies. He regards it as very probable that the phosphate of lime is laid down by the giant cell itself in the reaction against the bacillus. The final fate of the giant cells in this form of tuberculosis is not discussed by him.

The researches of recent years, carried out by Metchnikoff and his students at the Pasteur Institute in Paris, all warrant, according to their authors, conceptions quite similar to those first advanced by Metchnikoff concerning the giant cells, and the general conclusion of this s that it is of essential importance in the fight against tubercle bac large protoplasmic masses with many nuclei are formed, so t activity of the intracellular processes may be heightened beca bodies to be digested are very resistant. This would not appear all a strange process when we remember that plasmodia are obs generally in connection with cellular digestion in the lower as w the higher animals.

The modes of formation of giant cells, the many possible so their origin, their appearances, their relation to the bacillus of t losis and to other parasites, as well as to foreign bodies, the de tive changes that may occur in their interior, and many other i ing and important features have all been carefully and re studied. But the further definitive changes that may occur in t ments when the tubercle bacilli or other micro-organisms die or sorbed before the cells have undergone necrosis, or after the substances have been completely removed, have, strange to ceived but scant attention. Tuberculosis not infrequently heal lis heals, actinomycosis heals, and foreign bodies are often con absorbed. Are the giant cells, so generally present in these tions and which sometimes persist even after the active ca brought them into existence has been removed, capable of a gressive changes? Do they continue to exist as giant cells, complete healing or absorption mean their removal by way of gration? Manifestly a decisive solution of this rather neglec blem, in connection with the giant cells of tuberculous tis instance, would materially aid us in arriving at some definite sion concerning their nature, whether they are to be regarded a necrobiotic elements, or as structures possessing an independe ity that may outlast that of the tubercle bacillus, and in the tion of which they perhaps play an active rôle.

The exact histological details of healing non-degenerated t lous tissue have been lost sight of on account of the general sion that the healing is accomplished, in the main, through and necrobiosis of the tuberculous tissue, together with calci followed by more or less regeneration of the specific tissue elements, the principal part of the resulting defect being bridged over by cicatricial tissue.

That tubercle does not always undergo caseation was noted by Cruveilhier (quoted by Straus), who states that tubercles of the lungs, in particular, are curable at all periods of their development by means of a sort of fibroid encystment (tubercules de guérison). Again, Grancher states that all tuberculous granulations that develop slowly become fibrous and heal, that is, they are transformed into a sclerotic and inoffensive anatomical product in the form of a " fibro-caseous neoplasia."

D. J. Hamilton, in his Text-Book of Pathology, says that sometimes the tubercle does not caseate, but continues to live on and to develop into what seems to be its ultimate stage of organization. In or quite near to the centre is usually a giant cell, around which there may be found smaller giant cells. From the periphery of the giant cell processes come off that by subdivision produce a reticulum which is more condensed at the margins of the tubercle. In the meshes lie lymphoid corpuscles and one or more giant cells. This is the reticular tubercle first described by Wagner. As this tubercle becomes older the reticular giant-cell system is replaced by a simple mass of fibrous tissue; the giant cell becomes more and more fibrous at its periphery, and the protoplasm of the cell becomes transformed into or secretes the fibrous margin, which is covered with nuclei that appear to be derived from the giant cell. During this process of fibrous transformation the bacilli have vanished.

The investigators that have studied the action of dead tubercle bacilli upon the tissues make no statements as to the ultimate fate that befalls the giant cells that may form in the cell accumulations around the dead bacilli (Straus).

The precise manner of healing of tuberculous peritonitis after laparotomy has been made the subject of a considerable number of investigations, the results of which differ somewhat, the variations depending, it would seem, upon the stage of the process at which the operation was performed. Osler reports the results of the microscopic examination in the case of a woman who died of acute pneumonia 4½ months after a laparotomy which had been followed by marked improvement of the abdominal condition; the peritoneal tubercles were changed to fibrous masses containing giant cells with fatty granules, abundant bacilli, but no caseous material. Pichini, Bumm, Riva and D'Urso found that in healing tuberculous peritonitis after laparotomy (in man) the tubercles are replaced by fibrous tissue, and, according to Bumm and D'Urso, the giant cells are destroyed by wandering cells. Among those w studied the question experimentally may be mentioned Kichei Stchégoleff, both of whom regard the improvement and healing follow laparotomy in experimental tuberculous peritonitis in gui dogs and rabbits as due essentially to phagocytosis, to infiltrat round cells, to the formation of fibrous tissue while the "spe ments of the tubercle" disintegrate and undergo absorption. Nannotti and Baciocchi, and especially Gatti, emphasize drop generation of the epithelioid cells as an essential step in the Gatti, who studied this problem in guinea-pigs and rabbits, bı pally in dogs because of their greater resistance and of the mor chronicity of the tuberculosis, comes to the conclusion that th healing, macroscopic, histological, and biological, that unque does follow simple opening of the peritoneal cavity, when the of the peritoneum have reached a certain stage—the fibroid bu caseous—does not depend upon inflammatory reaction nor upon ation of the connective tissue, but upon a dropsical degenerati epithelioid cells, which melt together to form small spaces, nuclei are dissolved, and finally the remnants of these cells completely, together with the lymphoid cells, leaving simply existing stroma; fibroblasts and karyokinetic figures do not occı reasons that the poison that kills the epithelioid cells does n leucocytes; that the bloody serum, which forms after the la kills the bacilli or so weakens them that the resulting prote slow degeneration of the cells; and that, therefore, the prime the operative healing of the peritoneal tuberculosis is the exu serous fluid into the abdominal cavity. When the tubercles hav caseous laparotomy may arrest further caseation, but has no dir ence upon the removal of caseous masses.

It will have been observed that practically all of the autho fail to record any observations as to the exact changes in the g in healing tuberculous peritonitis—Gatti rarely mentions the g in his articles—and, furthermore, that none of the investigators any active part in the healing process to the cells of the tubercul itself, all speaking solely of degeneration, disintegration and a of the cells of the tubercles.

In his work, "Die causale Behandlung der Tuberculose," votes a chapter to the consideration of the histology of the heali cle, as observed in guinea-pigs suffering from experimental tu and treated more or less successfully with the products of the bacillus. He advances the view that the proliferation cause tubercle bacillus protects the bacillus against the bactericidal action of the fluids of the tissues, hence the latency of many local tuberculous infections. When the tuberculous tissue loses its protective properties on account of degenerative changes, the bacilli may die or they may multiply, depending upon various local and general conditions. Under the influence of the tuberculin and its purified modification, tuberculocidin, there is produced an exudation which deprives the cells of the tuberculous infiltration of their protective powers and the bacilli are killed by the serum. Now, the cells of tuberculous proliferations do not possess unlimited powers of proliferation, and hence tuberculous tissue is susceptible of healing. After the death of the bacilli, the tuberculous tissue may disappear partly by necrosis, regeneration and cicatrization, partly by transformation into mature tissue. While the capabilities of many of the cells are exhausted, so that they are merely useless elements that are best removed, tuberculous tissue may yet contain various cells of connective tissue as well as epithelial origin that possess an independent vitality and outlive the tubercle bacilli. Such cells may, in the course of further events, become component parts of apparently quite normal structures. Thus the giant cells in the alveoli of healing pulmonary tuberculous areas may subdivide into small uninuclear cells; the nuclei become richer in chromatin, karyokinetic figures form, and the cells apply themselves to the alveolar walls and become part and parcel of its lining. The cells that do not form, or are not needed to assist in the formation of the alveolar lining, are thrown out. In the healing of tubercles in the periportal tissue of the liver, the giant cells which, according to Klebs, are formed from the lining of the bile ducts, separate again into individual cells. In healing splenic tubercles of guinea-pigs the giant cells, which are all intravascular, become reduced in size by contraction of the protoplasm and remain for a long time after the disappearance of the bacilli, but Klebs thinks that they are finally divided up into endothelial cells. The giant cells in tubercles in the lymph nodes are formed by the endothelial cells of the lymph vessels, and may, after the destruction of the tubercle bacilli, separate and revert to endothelial cells again; such giant cells, therefore, are closely related to the intravascular giant cells.

Klebs regards the non-epithelial giant cells in connective tissue as coming from the angioblasts. He observed in a case of recurrent tuberculous pericarditis that the miliary tubercles were surrounded by wide vessels which passed into the tuberculous tissue, where they disappeared, their place, however, being taken by very large giant cells which were surrounded by round cells. The protoplasm and the processes of the giant cells stained more deeply with the ponceau dye than the si ing cells. The nuclei were arranged with their long axes vertic surface and usually around a clear centre. While not able to distinct and immediate connection between the giant cells and sels, Klebs seems to believe that as the vessels are compressed b lation tissue they may fall into fragments or pieces that remain fi various substances, that the endothelial cells multiply and the an form giant cells which may subsequently divide into endothel although the only evidence he has seen of this latter change found in human tuberculosis, but in the tuberculous spleen of pigs.

While studying the vascular changes in the leptomeninx i of clinically anomalous tuberculous leptomeningitis of an u long duration, my attention was directed to the relatively number of giant cells present and to certain peculiar chan sented by them, that seemed to point quite clearly to the oc of other than merely retrogressive processes in the specific p tions. I consequently utilized the opportunity to study more larly the giant cells of this form of healing meningitis. Th of this part of my work are embodied in this article; further tions made in connection with suitable experiments are rese publication later.

Tuberculous leptomeningitis may, like tuberculous inflar of other serous membranes, heal spontaneously. Dujardin-B Bauer, Sejournet, Weir, Nilsson, Martel, Klein, Freyhan, a many others, describe cases diagnosed clinically as tuberculou gitis which ended in recovery. Ord describes a case diag cerebral tuberculous meningitis in which recovery follow drainage through a trephine opening into the subdural space

While the diagnosis in such cases as the above may be ope cism, Fütterer examined post mortem a patient who had suff years before death from a disease diagnosed by Leube as spin culous meningitis, and found the spinal leptomeninx to shrunken, calcareous, miliary tubercles, *i. e.* he furnished the ical proof of a healed degenerating tuberculous leptomeningi

Atypical, subacute, and chronic, sometimes latent, forms culous meningitis also occur. Whereas, according to Strümpel, the average duration is between three days to one and one-half weeks, Seitz mentions two cases of forty-two and fifty-four days, Ludwig Meyer one of forty-nine days, Neudörffer one of fifty-six days, and Busse one which lasted at least sixty days. These cases occurred in adults.

In the case described by Busse, which occurred in a woman thirty-seven years old, the pia was adherent to the cortex and contained numerous flattened and hard elevations of irregular size and outline. In the Sylvian fissures were small, grey, translucent nodules. Caseation was absent. The areas of thickening consisted of interlacing bundles of fibrous tissue, containing here and there masses of cells and also occasional giant cells. Tubercle bacilli were found in small numbers inside as well as outside the giant cells. Evidently there was here a chronic tuberculous inflammation in process of arrest and healing.

The present case was that of a man, white, aged 25, admitted into the service of Dr. Norden, at the Cook County Hospital, December 13, 1895, with a history of having been sick for 35 days before admission with headache, some nausea and pain in the stomach. He had been working in a rolling mill before he became sick. He had never had any serious illness before and denied all venereal infection. Precise information could not be obtained because he was stupid and dazed.

Physical examination showed a medium-sized, rather slender and poorly nourished man; the organs of the chest and abdomen were quite normal. The tongue was dry and coated. There were some spots on the arms that were thought to be rather suspicious of syphilis. The cervical glands were enlarged. The temperature, pulse and respiration were normal at the time of admission. He was placed upon iodide of potassium, one gramme four times daily.

During the days succeeding his admission he complained at times of very severe headache, more so at night, and would clutch his head with his hands and moan. There were no focal cerebral symptoms. After a week or so the potassium iodide was increased to two grammes four times daily, and further notes of headache were not made. The record shows that while his pulse and respiration remained normal the temperature would occasionally mount up to 101° F. There was marked costiveness and much nausea, the appetite being poor. After December

30th his temperature but rarely rose above 98.5°. Examinati urine was negative except for hyaline casts. Examination of with the ophthalmoscope on February 15, 1896, by Dr. Wescot the disk to be poorly defined on the right side, less so on the tubercles were found in the iris.

The emaciation increased somewhat; the symptoms and signs monia appeared shortly before his death on March 6, about fou after the beginning of his sickness.

The clinical diagnosis was cerebral gumma and chronic neph

Post-mortem examination.—Ten hours after death.

Anatomical diagnosis.—Chronic tuberculous leptomeningitis tuberculosis of the peribronchial lymphatic glands; acute b lobar pneumonia; adhesive pleuritis; adhesive perihepatitis, itis and periappendicitis; chronic gastritis; parenchymatous venous angioma of the liver; decubitus.

The body is poorly nourished and of rather slender build. mortis is marked. There is a small bed-sore over the sacrum.

The peritoneal cavity is empty; there are adhesions between and the diaphragm and between the gall bladder, the duodenum colon, also around the spleen and around the vermiform appen

The pleural cavities are empty; adhesions are present posteri both lungs. The pharynx, larynx and trachea are normal. T chyma of the lungs is spongy and pale grey in color, except right lobe, which is solid, and rather homogeneous; from its cu which is of a red color, small whitish casts are scraped aw bronchi contain muco-pus. The peribronchial lymphatic gl enlarged, containing caseous areas, calcareous masses and small nodules.

The pericardium is empty and the surface of its layers is The heart weighs 240 grammes, the endocardium is smooth, tl uniformly firm and greyish brown in color. The aorta is smoo

The spleen weighs 200 grammes and on section is seen t much fibrous tissue.

The kidneys weigh 230 grammes together; the capsules are cortical markings distinct, the medullary rays being especially consistence firm. The ureters, seminal vesicles, and prostate ar

The liver weighs 1400 grammes; of firm consistence; near th surface of the left lobe is a venous angioma about 2 cm. in i diameter. The gall bladder is normal.

The œsophagus is normal. The mucous membrane of the s thickened and red. The large and small intestines are norn pancreas is normal.

The scalp, the skull and the dura are normal. At the base of the brain there is much turbid serous fluid and a thick layer of rather firm, translucent, gelatinoid material matting the structures together quite firmly. Along the arteries in both Sylvian fissures are numerous whitish and greyish yellow nodules, of pin-head size and larger, imbedded in the translucent material with which they seem to be infiltrated. The ventricles are dilated, containing much turbid fluid, the lining being rather soft. The brain substance is normal.

Both ears are normal; the sphenoidal sinuses are normal. The spinal cord was not examined.

Bacteriological examination.—The lungs were found to contain, in the pneumonic lobe, the Micrococcus lanceolatus, which was present in cover-glasses prepared from the exudate and was also isolated in the pure cultures.

The liver contained a coccus corresponding to the staphylococcus pyogenes albus, but cultures made from the spleen and the kidneys remained sterile.

Cover-glasses prepared from the meningeal exudate did not contain any micro-organisms when stained with ordinary dyes, and special stains for tubercle bacilli failed to reveal any bacilli (4 cover-glasses). A glycerine-agar tube inoculated with a liberal quantity of meningeal exudate remained sterile after having been in the thermostat for four days; at the end of this time it was sealed and returned to the thermostat, and on May 26, 1896, it was found that a pure growth of tubercle bacilli (mammalian) had developed in typical form.

Histological examination.—There is marked degeneration and desquamation of the epithelial cells lining the convoluted tubules of the renal cortex.

The changes in the leptomeninx and its vessels presented many features of interest and they were, therefore, extensively studied in serial sections prepared in the following manner: The tissue had been fixed and hardened in nearly absolute alcohol; small pieces from various parts of the basal leptomeninx, usually cut so as to have one or two vessels running through the centre of the longer diameter, were cleared in cedar oil and imbedded in paraffin; extensive ribbons of sections at right angles to the vessels were made and fastened on the slide with albumin fixative; the different series of sections would vary in thickness; some were 4μ, others 6μ, others 10μ, depending upon the exact minimal thickness at which perfect series could be obtained. The majority of the slides were stained with Delafield's hæmatoxylin; differentiated with 1 per cent. aqueous HCl solution, washed carefully and for a long time in water, dehydrated in absolute alcohol, cleared and counterstained wi cloves containing in solution enough eosin to give it a rich red excess of the oil being removed by xylol. Van Gieson's hæ and picric-acid-fuchsin method was also used, and a number of aside for the staining for tubercle bacilli were either colored wi toxylin, stained with carbolfuchsin and dehydrated with xylol-a after a short bath in the iodine-iodide-of-potassium solution, o directly with carbolfuchsin, decolorized with 20 per cent. sulph and counterstained with methylene blue.

The inability to obtain the ponceau dye, used and recomm Prof. Klebs for the purpose of counterstaining sections of heali culous tissue, prevented its employment.

The changes were now studied in the various series of sectio were found to include in a perfect manner very many larger an miliary tubercles; the appearances of the giant cells could be lowed through the whole thickness and extent of the cell.

The vascular changes.—The intima of the larger arteries at th the brain may show a more or less well-marked thickening, d presence of a sparsely nucleated fibrous tissue situated between t of the vessel and the tunica elastica. This thickening is best mar the adventitial and extravascular changes to be described are tense. In many instances this intimal thickening contains di hyaline degeneration which assume a faint bluish-violet ti stained with hæmatoxylin and eosin. The thickening in t arteries is usually semicircular in its extent and tapers from th central part towards the ends of the semicircle, the elastic laye being intact and the endothelial lining quite distinct.

The smaller arteries further out in the Sylvian fissures als more especially at points corresponding to the larger advent quite extensive chronic fibrous thickening of the intima that greatly narrows the lumen of the vessel. In some of the ser sections the deeper parts of the subendothelial fibrous tissue ar of advanced typical hyaline degeneration, but distinct caseati present. In other places the parts near the muscular coat are in cells, the amount of fibrillation being much less than in nearer the endothelial lining, so that here the tissue is compose or oval cells with quite deeply stained nuclei, lying in a ve meshed stroma. In one instance an indistinct giant-cell-like is recognized through three sections, each 5μ thick, is found lyi the fibroblasts (Fig. 5, Plate III); this cell is small and seems of a mass of very closely aggregated nuclei with a minimum cell body. In some places, again, the deeper parts of the proliferation contain spaces without any recognizable endothelial lining, but nevertheless filled with blood.

The endothelial lining over the thickenings is quite distinct and usually composed of small, closely set, rather typical cells. The cells in the layers immediately under the endothelium are not distinguishable, so far as shape and size are concerned, from the cells of the endothelial lining proper.

The elastic layer is everywhere intact, but the connections between it and the intimal thickening are apparently so loose and free that the thickened intima has often become more or less detached.

The extravascular changes.—The translucent material mentioned in the macroscopic description as surrounding the basilar vessels and other structures is composed, generally speaking, of a loosely arranged, richly cellular, very young fibrous connective tissue, throughout which are scattered a remarkably large number of variously shaped giant cells, often enclosed in small nodules of an indistinct concentric arrangement that might be considered as changed miliary tubercles. Caseation is almost absent; only in one or two places are small districts of caseation found in the adventitial foci of smaller arteries, and the caseous material seems to be enclosed in a capsule of more dense though quite cellular connective tissue. The adventitia of the smaller arteries in the Sylvian fissure and elsewhere present very small nodal enlargements made up of similar young fibrous tissue which also contains very many giant cells.

Many of the cranial nerves are the seat of interstitial changes similar to those above outlined.

The tissue referred to as young fibrous connective tissue may present a plainly fibrillated, sometimes lamellated, more often perhaps a very loosely arranged indistinctly fibrillated, granular or homogeneous matrix. Plain fibrillation, with occasional lamellation, is found at the periphery of the adventitial foci of the arteries, and this tissue is not so richly cellular; in such areas the endovascular thickening may be very pronounced. Mostly, however, this tissue consists of a matrix that stains more or less deeply reddish with eosin, being often composed of loosely and irregularly arranged bands of a fibrillar or granular structure of varying width and density; or the matrix may be quite homogeneous.

In this matrix lie various kinds of cells; usually the cells are scattered, but occasionally the cell infiltration is so marked that the intercellular substance can hardly be made out. In addition to the giant cells already mentioned, two other kinds of cells are distinguished: (1) Cells with rather large, lightly staining, occasionally almost vesicular

nuclei, that are oval, oblong, spindle-shaped, or of long draw
irregular forms. The cytoplasm of these cells often blends
intercellular substance so that the exact limits or shape of the
cannot always be made out; usually the cell body corresponds
in shape with that of the nucleus; it is finely granular, almost
ous and stains faintly with eosin. (2) Cells, small in size, with
large, round or oval, very deeply staining nuclei surrounded
rim of reddish cytoplasm; these cells, which are the most
would correspond, in many instances, in shape, size, and appe
the lymphocytes of the blood. There are foci and areas of ce
tion composed almost entirely of these cells.

In addition there are not a few cells which contain two n
indistinct or poorly preserved karyokinetic figures are foun
a while. Such cells cannot be recognized as belonging to eith
or the other of the two kinds of cells described above. The
cells that might be regarded as transition forms between
classes. Polymorphonuclear leucocytes are not present excep
sional single cells.

The tissue here described in general contains many small bl
the greater number of which are evidently of new formation.
capillary loops can be found in very many places, and the lo
the number of the vascular channels with thicker walls indicat
also are newly formed structures. The endothelial cells are
large and oval and are provided with swollen nuclei. It ma
at this point that while many of these vessels occur in close p
the giant cells to be described, yet the most thorough examina
series of sections has failed to disclose a single instance in
could say, with reasonable certainty of being correct, that the
played a direct part in the formation of the vessels. Direct
between the lumen of the vessel and the giant cell or its rem
not be demonstrated.

In some places the vessel wall is diffusely thickened and
vessels may appear almost completely obliterated.

Tubercle bacilli were conscientiously searched for in a large
suitably prepared specimens. In the sections stained with h
and carbolfuchsin some of the well preserved giant cells cont
sausage-shaped and irregular forms that are colored black an
somewhat the bodies described by Metchnikoff, Welcker an
changed and degenerate bacilli; but it would be difficult in
mens to differentiate between such masses and nuclear frag
some of the sections stained with carbolfuchsin and methyle

tween the cells are found small aggregations of bright red granular masses containing bodies not unlike cocci, and also larger irregular forms that may be altered tubercle bacilli or remnants thereof, as well as a very few quite typical bacilli; isolated bacilli were also found between the cells, but only in very occasional instances.

The giant cells (Figs. 1–4, Plate III).—Imbedded indiscriminately in the granulation tissue are, as stated, a remarkably large number of variously appearing multinucleated giant cells, which, speaking generally, seem well preserved and free from evidences of extensive degeneration. The giant cells may in some instances seem small and appear immediately surrounded by ordinary young connective tissue; or they may lie in small cavities, as it were, a distinct though irregular space separating the cell from the surrounding tissue; more frequently, perhaps, they are immediately encircled by very loosely arranged cells with a tendency to a concentric arrangement, the whole resembling to a certain extent, on first view, a miliary tubercle; there are also small foci occupied by loosely scattered cells with an imperfect, concentric peripheral disposition, producing sometimes an indistinct central lumen or space, which may be quite empty, or in which, as well as in its walls, may lie giant-cell remnants, nuclear fragments, and endothelioid nuclei imbedded in protoplasmic masses similar to those of the fragmented giant cells with which they may or may not be connected.

The protoplasm of the giant cells is often almost homogeneous, or it may be uniformly finely or, in parts, more coarsely granular; the more coarsely granular parts are, as a rule, found near the centre of the cells and may contain, when more extensive, irregular chromatic fragments, while the surrounding nuclei may stain quite well. Generally the body of the giant cells sends out irregular and branching prolongations that pass among the surrounding cells and seem to fuse inseparably with the homogeneous or granular intercellular substance; at times, however, the outlines of the cell are very sharp, distinct and regular. Under all circumstances, but more particularly in the latter instances, the main protoplasm of the giant cells stains a little more deeply with eosin than the intercellular substance, and on this account it is possible to trace the origin of the many larger and smaller multinucleated and uninucleated fragments into which the giant cells are often separated. In many instances the giant cells are vacuolated; those vacuoles are produced in part by nuclei that stain very faintly; especially is this the case when the borders of the nucleus only take the stain, the vacuoles being then quite distinct in spite of their small size. In part the vacuoles seem to result from the disappearance of the protoplasm of the giant cell immediately about round nuclei, which are often deeply stained; t uoles sometimes contain nuclear detritus and occasionally they a large (Fig. 4 B, Plate III).

The size and the outline of the giant cells naturally vary mu the various series of sections the majority of the cells can be through a very considerable number of specimens, and in this wa be estimated that in some diameters many cells may equal as n 60, 80, 100 and even 200μ or more; it may also happen that th diameters are not very much less, and consequently in some sect giant cells are almost recognizable with the naked eye. On acc the branching prolongations usually present, the outline, as a rul irregular. At the periphery of many cells, and especially at th there are often found scattered cells and cell masses that have nu protoplasm similar to those of the main giant cell, and that cons are either in the act of fusion with or separation from the giant

The number and the arrangement of the nuclei also vary grea are observed with probably hundreds of nuclei. In a large ma the comparatively unchanged cells studied, the nuclei are arr the manner characteristic of the typical Langhans giant cells, a a more or less complete circle near the periphery of the main pa body of the cell. They often seem to have the long axis vertic surface of the cell. The nuclei are usually quite small, oval or shaped; they are often rather poor in chromatin substance a somewhat faintly; not infrequently they are vesicular, and w the borders of the nucleus stain, and that rather faintly, the nuc appear like a distinct vacuole; at other times the giant cells con or round nuclei that are rich in chromatin and stain deeply. T also frequently present very atypical forms; they may be d shaped, flask-shaped, or very long and drawn out; budding proc nected with the main nucleus by a slender thread or stem are als and irregular, bizarre shapes are not infreqeunt. Occasionally preserved karyokinetic figures are also found in a single nuc giant cell. In some cells there are also found, as already state fragments and detritus.

While the above description would answer in a general closer examination with the high powers of the microscope re many other interesting appearances in the nuclei and the prot the giant cells that, although presenting difficulties in the w cise and systematic description, as well as satisfactory inte nevertheless merit some attempt thereat.

Division of the giant cells with nuclear disintegration.—It mentioned that a considerable number of the giant cells seem to be in the course of division into or, possibly though not probably, fusion with smaller multinucleated masses and uninuclear cells. At times this process is associated with the formation of more or less detritus of nuclear origin and a splitting up of the cytoplasm into more minute fragments, together with the appearance of cells or phagocytes that are loaded with the remnants. Such manifestations are most noticeable in the vicinity of foci of caseation, but they are also found elsewhere. It is also observable that the bodies of the cells and cell fragments under these conditions are usually more coarsely granular. In many cases the giant cells seem to have split up into two or more separate and unequal parts, and the centre of the place that was occupied originally by the giant cell may be strewn over with nuclear fragments, occasional polymorphonuclear leucocytes and other cells filled with detritus. In other places the interior of the still intact or partially fragmented giant cell may contain much nuclear detritus, as well as vesicular and more deeply stained nuclei, while there may be arranged at its periphery cells with deeply staining nuclei which lie in more or less distinct lacunæ in the surface of the cell that then has a scalloped appearance; in a few cases these cells seemed to contain in their interior smaller and larger pieces like the substance of the body of the giant cell. In this way there may be formed, as it is easy to trace in the serial sections, a small area in which the matrix stains brighter red than that of the tissue immediately around it; the area is occupied by rather loosely scattered cells with the appearance of a concentric arrangement, especially at the periphery, producing here and there an indistinct, irregular central space or small cavity without any direct connection, however—and this was looked for very closely—with the blood-vessels. The small cells are usually oval, curved, spindle-shaped, or irregular, with a reddish, finely granular, frequently indistinct protoplasm and spindle-shaped or oval, quite lightly stained nuclei; distributed among these cells may be other round or oval cells with more chromatic nuclei and also a varying amount of free and intracellular small, deeply colored nuclear fragments, often shaped like cocci or rods; furthermore, there are not infrequently found irregular portions or remnants of the giant cells with typical or with very peculiar, flask-shaped, club- and dumb-bell-shaped nuclei, and also cytoplasmic fragments without any nuclei; polymorphonuclear leucocytes are present in rather insignificant numbers, but more particularly when the disintegration is rather well marked. Often such a mass as above outlined may be surrounded by a small capsule of a more condensed tissue, and under the low power many of them may resemble miliary tubercles.

In the above described process the amount of nuclear disinte the irregular splitting up of the giant cells, the coarseness of gra of their protoplasm, and the presence of phagocytes all indicate t is dealing with structures that are undergoing retrogressive chan disintegration. But even in these cells there are abundant evid the fact that not all the nuclei of a partly necrotic giant cell are to destruction. In many places the appearances point to a direct tion of apparently viable cells from the giant cells; the nucleu part wholly or partly separated may become rich in chromatin a deeply; one can also observe that the nuclei in the interior of a grating giant cell, or of a fragment thereof, become more deeply surrounded with a faint rim of protoplasm outside of which a with a faint network may form (Fig. 4 A, Plate III); now, when a of nuclei undergoing such a change as this are arranged across cell there may result a complete separation of the cell into unequ during the time that some nuclei in the giant cell are undergo gressive changes, others have suffered disintegration, as show irregular chromatin fragments scattered about. At the poles cumference of such giant cells there are also frequently found g more or less deeply stained nuclei, surrounded by a small an protoplasm, that give the impression of having become separa the giant cell.

Division of giant cells without much disintegration (Figs. Plate III).—There remain to be described a large number of g which seem to have separated, or to be separating, into a numbe cells with but slight or no evidences of concomitant nuclear nec disintegration of the protoplasm.

In quite a number of giant cells, with a finely granular cytop a more or less perfect peripheral wall of rather faintly staine there is found a varying number of deeply stained round nuclei which may lie in vacuoles in the cell substance, others upon t plasmic processes of the giant cell, while still others may be immediately outside the cell and provided with a cell body Plate III). An apparent connection between the lightly a stained nuclei can be traced through transition stages. Rath preserved karyokinetic figures are also present in a very few cells; budding processes are also seen arising from some of t Towards the periphery and the poles of such a cell there are fo a number of deeply stained nuclei with rather indefinite cell b still enclosed within the body of the old giant cell. The nucl of the new cells, especially the ones that are free, may assume shaped form. There are no appearances indicating that cells are in the act of wandering into the giant cell from without. Polymorphonuclear leucocytes are not, as a rule, seen in the vicinity of giant cells undergoing such changes as these, and there are but few nuclear fragments. When these changes have advanced throughout the whole giant cell, or throughout a whole segment of one, there is formed a focus of loosely scattered oval and spindle-shaped cells with deep or lightly stained nuclei, occasionally presenting mitotic figures, and with indistinct cell bodies situated in a loosely meshed stroma; immediately around such a centre may be a more closely arranged wall composed of cells imbedded in a distinctly fibrous matrix. In general, these areas, in some part of which portions of the giant cell are usually still present, remain distinguishable from the surrounding tissue also by the fact that they have a more reddish intercellular substance, that they are not so cellular and that the nuclei of the cells are less deeply stained. In the most advanced districts, where the fibrillation of the young connective tissue is most marked and the cellular infiltration less pronounced, the areas described are usually small and wholly occupied by cells, many of which cannot be distinguished, as regards shape, from the cells in the recent fibrous connective tissue around the area. Appearances indicating the transformation into blood-vessels of such districts of cells derived from the disunited giant cells are not present.

Undoubtedly the loosely filled spaces above referred to would later on become filled or obliterated in part, perhaps, by cell proliferation, but principally, most likely, by contraction of the intercellular substance.

SUMMARY.

From the foregoing description of the histological changes in the leptomeninx it is quite evident that we are dealing with a chronic, stationary, healing form of tuberculous inflammation.

This statement is substantiated, in the first place, by the clinical history. The only reasonable interpretation of the symptoms would establish the duration of the process as four months. The imaginable contingency that there existed first a meningeal syphilitic lesion that was dispersed by the iodide of potassium only to be followed by a tuberculous infection is so remote and unlikely that it need not be discussed. At all events the tuberculous leptomeningitis, which presented a typical distribution, began insidiously, existed at times in a latent condition, and pursued a very anomalous course, marked by a relative mildness of all the symptoms, and thus it came abou when an apparent or real improvement followed the administrat iodide of potassium able observers were induced to make an errc diagnosis. Death occurred as a result of an intercurrent infect

The long duration of the process is also shown, anatomical the thick layer of firm, translucent and gelatinous material that ı together the structures at the base, and also by the evident adl between the pia and the brain.

The histological examination furnishes proof positive of the c ness of the conclusion in regard to the peculiar character of th cess because it shows:

(1) That the tuberculous proliferation is uniform in develc and has reached nearly the same stage of evolution throughc entire extent of the leptomeninx involved; it is not a process t advanced by exacerbations and irregular extensions; the lesio generally speaking, of nearly the same age everywhere and mu begun at about the same time.

(2) That only a very limited degree of caseous degener present, pointing to an early arrest of the activity of the t bacillus or to a very decided diminution or attenuation of its vi

(3) That the subendothelial intimal proliferations of epi cells, so generally found in acute tuberculous leptomeningitis in this case become more or less completely changed into fibrous tissue in which but very slight, if any, direct evidenc tuberculous origin can be found. It is only by recognizing chronic endarteritis is most marked in correspondence with t advanced adventitial tuberculous changes, and by finding aı fect, much altered giant cell in one district of intimal thickeni we were able to establish the direct kinship of the endo changes with those of the pia in general.

(4) That acute inflammatory changes, in the form of en of polymorphonuclear leucocytes and of fibrinous exudation,

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* See an article by the writer entitled "The Vascular Changes culous Leptomeningitis, especially the Tuberculous Endarteritis, Journal, Vol. I, No. 1, 1896.

tirely absent in all parts of the district involved. The presence of a turbid serous fluid is of course not at all inconsistent with the view that the anatomical changes are of long duration.

(5) That the granulation tissue present is, in general, undergoing fibrillation and contains a rich supply of embryonal capillary vessels as well as of larger blood-vessels of evidently new formation. The absence of any considerable extent of polymorphonuclear leucocytic infiltration in this tissue has already been referred to. The cells in the granulation tissue correspond to the cells of embryonal or formative connective tissue. Vacuolation is rarely present.

(6) That the unusually large number of giant cells present are remarkably free from evidences of necrosis and degeneration of the character ordinarily observed in tuberculous proliferations, that they do not contain in demonstrable form tubercle bacilli, and that the majority of the giant cells seem to be separating into individual cells and smaller masses often with, but sometimes also without, evidences of nuclear disintegration. The possibility that these phenomena may signify fusion instead of the sundering of cells will be discussed below.

For these reasons there can be no doubt that the general claim that we are dealing with an instance of chronic, healing tuberculous meningitis must be regarded as established beyond dispute.

The growth of tubercle bacilli in the glycerine-agar tubes, inoculated with the fluid from the pial meshes, and the demonstration of tubercle bacilli, though in very small numbers, between the cells of the embryonal tissue, furnish the positive evidence that we are actually dealing with a tuberculous process due to living and not to dead bacilli.

The degree of virulence of the cultures of tubercle bacilli was, unfortunately perhaps, not studied. The presence of living tubercle bacilli in a tissue free from active and acute changes characteristic of tuberculosis demonstrates that, whatever the actual degree of virulence of the bacilli may have been, the tissue in which they were found was at this time relatively immune from their action. The manner in which this immunity was produced, and in which the process of healing was initiated, need not be discussed at this time any further than to again direct attention to the fact that the bacilli lost

their virulency as regards the cells in this leptomeninx befor
cells underwent any marked degree of degeneration. The cell
tuberculous proliferations survived the further action of the
whose original effect it was to initiate cell accumulation or pr
tion; the cells also retained sufficient vitality to develop, in s
stances at any rate, into formative cells according as their origi
dictate, *e. g.* into fibroblasts. That fibroblasts are formed
embryonal connective tissue cells, and not by wandering cells,
the large mononuclear leucocytes, we are well aware, is possi
a disputable assumption, and we do not consider it pertinent t
the question any further in connection with this study, but wo
emphasize the point that some of the cells of tuberculous p
tions may, under favorable circumstances, become formative ce
furthermore, that the amount of formative tissue produced
far in excess of what is actually needed for purposes of rep
Surely the appearances here noted indicate that the bacillus
culosis has the power to stimulate fixed cells to multiply, u
assumes that all, or almost all, the formative cells here seen
rived from wandering cells attracted by the presence of the
and its products.

As to the ultimate fate of the formative and other cells in t
ing tuberculous tissue no final statements can be made. It
remembered that it is only one stage in the process of healin
dealt with. The well marked evidences of fibrillation, the
tensive formation of new vessels, the absence of evidences of
tive changes in the uninuclear cells, all point to the productio
fibrous tissue as sure to occur, but it seems quite probable t
sional epithelioid cells may undergo or have undergone dr
other forms of degeneration, although it is certainly appare
far as the small cells are concerned the involution of the tu
tissue is not occurring through disintegration.

Perhaps the most interesting feature in this case is the op
it affords to study the changes in the giant cells of healing
generated tuberculous tissue. In the first place, the large
giant cells is quite remarkable. The general characters of

in which they are found recall the fact that giant cells are regarded as quite constant elements in chronic mild tuberculosis; often the giant cells are the only cells that contain bacilli (Koch). In this instance the giant cells do not contain bacilli that are demonstrable by the usual methods; neither do they contain bodies that can be definitely interpreted as degenerate forms of bacilli such as those found by Metchnikoff, Stchastny, Welcker, and others, in the giant cells of Spermophilus guttatus, in avian and in human tuberculosis. Metchnikoff states, however, that he knows of the occurrence of such degenerate forms only in the Spermophilus guttatus under the circumstances mentioned, and in the rabbit and guinea-pig in mammalian tuberculosis, but not in man; consequently, the manner in which the giant cells rid themselves of the bacilli undoubtedly present in their interior at some time during their existence, must as yet remain without any explanation.

In the description of the histological changes the various appearances presented by the giant cells are described somewhat minutely. The essential observations made concern, in my opinion, the further fate of giant cells which are still found to persist in healing non-degenerated tuberculous tissue. It was, I believe, quite conclusively shown that the consecutive changes appear to consist in the breaking up of the nuclei, the removal of the detritus by phagocytes, and the formation of a few apparently viable uninuclear cells in the case of more degenerated, exhausted giant cells, while other, and, as it would seem, better preserved or younger giant cells, separate into a number of individual, uninuclear cells with but little or no nuclear disintegration.

Objection might be raised to this interpretation of the appearances in the giant cells. While no one could very well dispute the view that part of the giant cells are undergoing retrogressive and absorptive changes with the production of some viable cells, a question might well be raised concerning the nature of the process taking place in those giant cells that have been spoken of as splitting up or dividing into uninuclear cells and smaller multinucleated masses without much evidence of nuclear disintegration. It might be claimed that the process is one of fusion of many cells to form giant cells, and not division of fully formed giant cells into small cells. But view of the processes described speaks against fusion. In place we are not dealing with a stage of tuberculous proli (Baumgarten), or cell accumulation (Metchnikoff), in wh would look for the production of giant cells, no matter whi concerning the histogenesis of tubercle be assumed as the corr because it has been demonstrated that, from whichever point the lesions are examined, the same positive conclusion that in the process of healing is reached; there is, therefore, no for the formation of new giant cells in such wide-spread throughout the district involved.

It might be claimed that the cells became arrested and, as fixed in the act of fusion which was taking place in the ea of the meningitis, but it would be difficult to understand the the stimulus that could hold the cells together in such a pecu ner for such a long time. It must be remembered that bacillary detritus could not be found among the incomplete complete giant cells.

In the second place the difference between the cells that a going disintegration and those regarded as dividing is essenti certain extent at any rate, one of degree, because in the first there is not much, if any, doubt but that viable smaller cell formed, and in the second instance some, though often ve evidence of nuclear fragmentation is nearly always present; also be correct to infer that in advanced subdivision of a much, and perhaps all, of the nuclear detritus produced mi been removed up to the last trace; finally, the two extreme changes in the giant cells are connected by transition stages gradation from the one to the other. Hence it is justifiab clude, for the time being, that *in healing non-degenerated tissue, the multinucleated giant cells may in part disinte undergo absorption, in part form viable small cells; that changes may, and usually do, affect the same cell, but that in of cells—presumably the older or the more exhausted—the* *sive process is predominant, while in a second class of cells—presumably the young and vigorous—the progressive changes are the more marked.*

In this connection it may be pointed out that while there cannot very well be any question but that we are dealing only with dividing and not coalescing cells, yet if this conclusion should be disputed and found incorrect, then the only remaining alternative would be to infer that this tissue furnished a unique and striking example of the formation of plasmodial masses by fusion in human tuberculosis, a conclusion to which many pathologists would refuse to subscribe, if for no other reason than because it is not in accordance with the almost universally accepted teachings of Baumgarten and Weigert in regard to the mode of formation of the giant cells in tuberculosis.

Believing as I do that the giant cells under consideration are in the act of division and not at all of fusion, there remain to be discussed some of the histological and other features presented by the dividing cells.

Many of the giant cells, perhaps the majority, contain larger and smaller vacuoles in the protoplasm. The exact significance of this vacuolation is not always clear. When the vacuolation accompanies an evident solution of the nucleus (karyolysis), there cannot be any doubt but that we are in the presence of a distinctly retrogressive process. Vacuoles are also most numerous in the giant cells that present other evidences of degeneration, such as coarseness of the granules in the protoplasm and extensive nuclear disintegration, but they occur as well around nuclei that stain deeply, around cells that seem to be separating from the giant cell, and even about nuclei that present mitoses. The formation of vacuoles seems to be responsible, to a certain extent at any rate, for the diminution in the volume of disintegrating and dividing giant cells, as shown by the clear spaces that form about them; these spaces are too large and occur too uniformly to be attributed solely to artificial shrinking produced by the hardening in alcohol.

Further undoubted evidence of retrogression in certain giant cells is the occurrence of nuclear disintegration, or karyorhexis, which sets free larger and smaller chromatin masses that are recognize giant cell as well as in the interior of the phagocytes usual around such cells.

Almost all the polymorphonuclear leucocytes found in tl are met with around giant cells with broken-up nuclei. nuclei of disintegrating giant cells can be noted appearances respond well to certain stages in the complicated karyorhexis in anæmic necrosis by Schmaus and Albrecht; some of the n budding processes correspond particularly well with those in their drawings; the interior of giant cells of tuberculous tis it would seem, present conditions favorable to the developme series of postnecrotic nuclear change. Vacuolation, karyo karyorhexis are the essential steps that lead to destruction of or parts of some of the giant cells; associated with these proce is usually observed a splitting up of the body of the giant irregular fragments with as well as without nuclei; and, as more or less phagocytosis of the resulting remnants of variou seen.

But evident degenerative and necrotic processes in a giant be associated with progressive changes. While some nuclei vacuolation or break up, others seem to become richer in and to stain more deeply at the same time that they seem cell bodies quite distinct from the protoplasm of the giant hyperchromatosis does not, therefore, seem to be a stage rhexis. A very few but undoubted karyokinetic figures we together with evidences of division of the cell body formed in cell protoplasm. Precisely similar changes are described by healing pulmonary tuberculosis of the guinea-pig; the nuc giant cells became rich in chromatin and karyokinetic figures Krückmann among others has found occasional mitoses in around foreign bodies, as well as elsewhere, but it would such mitoses have always been interpreted as indicating the mode of formation of the giant cells rather than of their i

The question of mitosis in existing multinucleated cells ha been studied by Krompecher, who concludes that the individ of such cells may undoubtedly divide by mitosis, either simultaneously or at separate times. Division by amitosis can also occur, but mitosis is the only progressive form of division, amitosis being a retrogressive, disintegrating process that must be looked upon as an evidence of degeneration of the nucleus. Ziegler states that in division of giant cells whose nuclei have multiplied by mitosis it may happen that the separating cell remains enclosed in the protoplasm of the mother cell. A singular phase in the involution of the giant cells in this pia is to be found in the existence of progressive changes side by side with nuclear necrosis and with degeneration; this finding indicates that giant cells may contain many independent elements which, though apparently fused into one large cell, may preserve their individuality so that while some nuclei die, others proliferate and perhaps feed on the remnants of their dead brethren and form new, viable small cells. The nuclei in giant cells may be looked upon as representing independent centres, capable at times of existing even though the cell protoplasm is disintegrated. Many of the giant cells separate into individual cells, unaccompanied or unassociated with much evidence of necrosis. These cells may be regarded as the more vigorous forms. Here also are observed occasional mitoses—but on the whole extremely few—and very constantly an evident increase in the amount of chromatin in the nuclei of the new cells as compared with the amount ordinarily found in the nuclei of giant cells.

These deductions concerning the persistence of the vitality of some of the nuclei, even in the presence of molecular and morphological changes in the cytoplasm and in other nuclei of the giant cell that lead to disintegration, are not entirely without the support of previous observations on cells, which, although made under different conditions, are nevertheless, it would seem, applicable to cells in general. Thus the brilliant investigations of Loeb upon the effects of various unfavorable surroundings, such as absence of oxygen or reduction of the amount of water, upon the cleavage of eggs of many kinds, show that the conditions which arrest development are qualitatively alike for nucleus and protoplasm, but quantitatively less for the protoplasm; when the irritability of the protoplasm is suspended the nucleus may segment without segmentation of the protoplasm, but upon r lishment of favorable conditions the protoplasm may divide int as many spheres as there are nuclei preformed—the nucleus p preserves the irritability of the cell and stimulates the protopl segmentation. From the appearances of the giant cells here de it would seem, then, that some nuclei are able to maintain their longer than others in the same cell, and under certain condit stimulate parts of the protoplasm to segment; in other cells nuclei have, as a rule, preserved their irritability.

The groups of cells formed by the dividing of the giant ce be traced by studying the process at the different stages in the di parts of the tissue. They assume an oval or spindle-shaped for coming more and more like the formative and endothelioid c young connective tissue, but their ultimate fate cannot be deter because it concerns essentially only one limited period in the i tion of the tissue. It may be said with reasonable certainty, ho that the new cells do not form blood-vessels, but as regards forming lymph-vessels nothing definite can be concluded.

It would not be safe to draw any definite conclusions, from t pearances described, with regard to the origin and the mode of f tion of the giant cells. The resulting small cells in general res very much endothelial and formative cells, but some of them a certain stages at any rate, not unlike large mononuclear leuco their final fully developed or mature condition being unknow positive inference can be drawn as to their pre-giant-cell origin. evidence points to the fact that the most probable origin of the cells, as indicated by their form and the apparent future career of descendants, would be the fixed mesoblastic cells of the pia. I gard to the mode of formation of the giant cells it is quite clea it must involve some process which is not incompatible with th bility of the small cells which may spring from the giant Whether this would speak more in favor of formation by fusion by karyokinesis of a single cell without division of the cell body not be well determined, ånd as long as authors are not agreed the question of the production of living, procreative cells by am

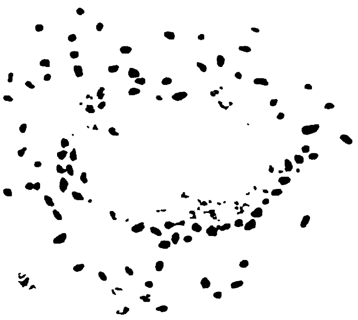

FIG. 1.

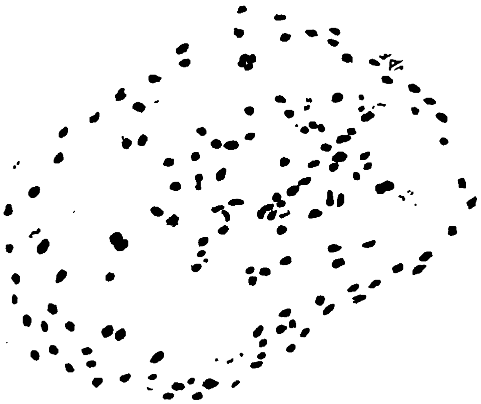

FIG. 2

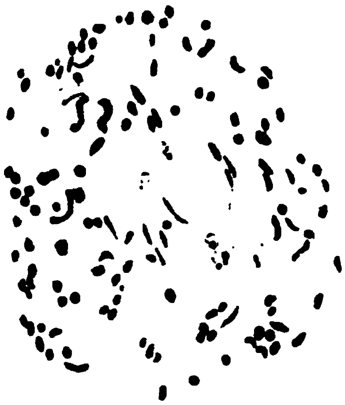

FIG. 3.

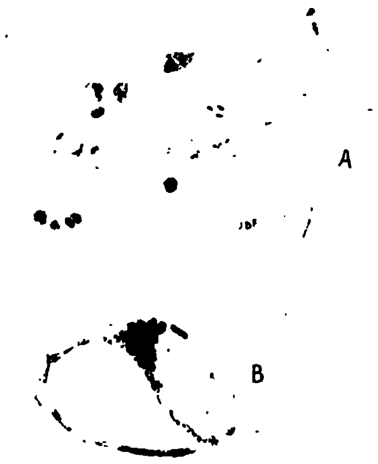

FIG. 4.

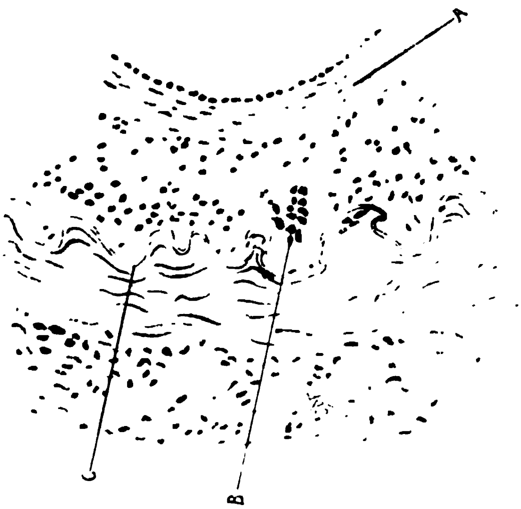

FIG. 5.

(direct segmentation, direct and indirect fragmentation) it would not be profitable to discuss the compatibility or incompatibility of the views of those investigators who trace the origin of giant cells to amitotic division, with the progressive changes that giant cells have been shown to be capable of.

The fact that giant cells in tuberculous tissue, under certain conditions, undergo progressive changes and separate into small, living cells proves that they are not, as claimed by Baumgarten, Weigert and others, necrobiotic elements that are doomed to destruction from their very inception. On the other hand it lends more strength, if that were necessary, to the teleological view urged by Metchnikoff that they are living, defensive cells (whatever their origin may be), formed for the distinct purpose, like plasmodial masses in general, of isolating and removing foreign, harmful bodies, in this case the tubercle bacillus, and, having accomplished their object without being destroyed or exhausted, or the cause of their formation being removed or neutralized in some way, they, or their nuclei, may retain enough irritability to form a larger or smaller number of living, small, uninuclear cells.

I wish to thank my friend Dr. J. D. Freeman for his excellent drawings.

DESCRIPTION OF PLATE III.

Fig. 1.—A giant cell in one portion of which many of the peripheral nuclei are rich in chromatin and stain deeply; there are vacuoles about some of these nuclei. To the left is a small fragment that has separated from the giant cell proper and which contains a few deeply staining nuclei as well as an indistinct cell with two nuclei. Zeiss, obj. 4°, ap. 0.95 apochr.; oc. 8 (compensat.); tube-length 160.

Fig. 2.—The same giant cell as in Fig. 1, but at a different level. The cell is separating into uni- and multinuclear cells and masses of varying forms. Magnification same as Fig. 1.

Fig. 3.—In the central part of the drawing are spindle-shaped and irregular cells and masses derived from the splitting up of a giant cell, as shown by study of the serial sections. Same as Fig. 1.

Fig. 4.—*A*, a section of an irregular, finely granular giant cell with faintly stained and vesicular nuclei, some chromatin granules, occasional deeply staining nuclei; and lying in a vacuole a distinct cell, the nucleus of which seems to have divided and the cell body of which presents a central constriction as though about to divide. At the lower margin of the cell are

separated protoplasmic masses, one of which contains a deepl nucleus.

***B*, giant cell with huge vacuoles, containing fine granules. Same**

Fig. 5.—Chronic fibrous thickening of the intima.

***A*, subendothelial fibrous tissue covered by closely arranged nucl**

***B*, imperfect or dividing giant cell (?) in the deeper, more cell of the thickening.**

***C*, intact elastic layer.**

Adventitia the seat of considerable infiltration. Zeiss, apochr. ol 0.95; oc. 4 (compensat.).

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A CASE OF PSEUDO-LUPUS VULGARIS CAUSED BY A BLASTOMYCES.

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PLATES IV–VIII.

Within the last three years a number of important communications have appeared concerning pathogenic yeasts. Before this period it was generally believed that among the yeast plants there existed none pathogenic for man or animals. The experiments of Bernard, Grohe, Popoff, Falk, and the more recent ones of Neumayer and of Raum, furnished no satisfactory evidence in favor of the possession of infectious properties by the blastomycetes.

In August, 1894, Busse * published a brief report, followed in April, 1895, by a fuller account of a case interpreted by him as one of chronic pyæmia, in which he found in tissue removed during life and in diseased foci after death, numerous parasitic bodies which he proved to be yeast fungi. These he isolated in pure culture and inoculated successfully into animals. He designated the disease produced by this parasite in man as saccharomycosis hominis.

In May, 1894, three months before Busse's first publication, one of us (Gilchrist) exhibited and described before the American Dermatological Association in Washington, microscopical sections from a case of cutaneous disease in Philadelphia under the care of Dr. Duhring, who had excised and sent a piece for microscopical examination. Peculiar parasitic bodies, which were interpreted as plant rather than animal forms, were observed and described. After the appearance of Busse's paper these bodies were recognized as forms of blastomycetes. This case has been fully described and illustrated by Gilchrist under the title of "A Case of Blastomycetic Dermatitis in Man."

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* References to literature will be found at the end of this article.

The most prolific writer upon the subject of pathogenic yea the year 1894 has been Sanfelice, who has obtained from sources cultures of blastomycetes pathogenic for animals. matic experimental investigation of the pathogenic properti animals of yeast cultures obtained from various sources has bee also by Rabinowitsch. The Italian investigators especially, of besides Sanfelice, may be mentioned Maffucci and Sirleo, Aievoli, Corselli and Frisco, Fermi and Aruch, d'Anna, Secchi, Rossi Doria, Casagrandi and de Simoni, have in themselves in this subject. Many of these writers, as well as in Vienna, deal with the question of the parasitic origin of nant tumors. Although it cannot be doubted that in some ir yeasts have been cultivated from such tumors by Roncal selli and Frisco, and others, we do not consider as thus far su the efforts to identify the much discussed cell-enclosures in car and sarcoma with blastomycetes or other genuine parasites.

Of interest are the observations of Tokishige concerning th tomycetic origin of a farcy-like disease of horses and cattle in apparently identical with a similar disease in Italy and Fran wise referred to blastomycetes by Fermi and Aruch, and also t Sanfelice concerning so-called epithelioma contagiosum (Bollin fowls, which had been attributed by Rivolta and Delprato to sperms, but which Sanfelice believes to be caused by a yeast p

Curtis, in France, has published interesting observations yeast parasite, which he calls Saccharomyces subcutaneus tumef found in a tumor of myxomatous appearance in the thigh of a being. This he cultivated and inoculated successfully into rats and dogs.

A preliminary account of the case which forms the subject present paper was published in the Bulletin of the Johns H Hospital in July, 1896. Since that time we have had the oppor of making more extended observations and inoculation experi and are, therefore, now in a position to give a full description case and the results of our investigations. The patient first under the care of Professor Halsted of the Johns Hopkins Hospit whose permission one of us (Gilchrist) had the opportunity of examining and studying the case. We take this opportunity of thanking Professor Halsted and also of expressing our indebtedness to Professors Welch and Flexner for their valuable aid and advice in our investigations.

Clinical History.—The patient is 33 years of age, married, about 5 feet 9 inches in height, and of a slender but wiry build. He is one of thirteen children, twelve of whom are still living and in good health; one child died when five months old from whooping-cough.

On July 4, 1885, the present eruption is said to have been first noticed at the back of the left ear, just behind the mastoid process, as a pimple which was about as large as a grain of wheat. It was roundish in shape and only slightly raised; the pimple soon became pustular. The eruption began to spread in a linear direction towards the lobe of the ear, but increased in extent very slowly, so that during the succeeding four or five years the length of the patch was only from 3 to 5 cm. As the disease extended the oldest portion gradually healed spontaneously, and a white, slightly hypertrophic scar was produced. The healing process was always only partial and took years for its accomplishment. The linear scar, which is now distinctly visible, is about 4 cm. long and 5 to 6 mm. broad.

After four or five years the disease, which continued to spread very slowly forward, began to involve the cheek, and then gradually extended upwards and downwards, so that seven years after the first appearance of the eruption it had only reached the external canthus of the left eye, after which it continued to travel along the eyelids. The tendency to heal as it progressed was still a characteristic feature of the process, but the scar was now more atrophic, whitish and thin, particularly over the cheek. Besides extending to the eye, the lesion also spread gradually down the left cheek until it reached the left side of the chin. In about nine years the nose was attacked by the growing edge; the bridge was soon crossed and the right eyelids and eyebrow became involved. After this the patient noticed that the growth became more rapid, especially on the forehead and down the right cheek.

About one month after the first lesion was observed there appeared on the back of the right hand, over the middle of the third metacarpal bone, another pimple which soon became pustular and opened spontaneously. This lesion also began to increase slowly; it remained superficial and assumed the same characters as that on the face; it took about four years for it to spread over the whole posterior surface of the hand, from the knuckles to the wrist, and from the thumb to the inner marg hand. The eruption was treated successfully with caustics by t physician.

Another secondary lesion occurred six months after the firs right side of the scrotum at the upper portion, and this agai stated, had the appearance of a pimple which followed the sa as the others, except that it contained only a little pus; but th teristic creeping propensities were shown until the area attained the size of a silver dollar. This scrotal lesion grew slowly fo when it healed spontaneously. No external applications were u

Still a fourth lesion appeared in the same manner and at a same time as the scrotal eruption, on the anterior surface of thigh and just above the internal condyle. This also spread p ally for about a year until it formed a patch 5 cm. by 7.5 cm. when it also healed spontaneously without treatment, leaving a raised scar. On the back of the neck, just above the 7th cervic a fifth lesion made its appearance a little later than the one on t and continued to grow for two years, when it also healed sponta The patient says none of the lesions were at all painful. Upon e of the patient into the Hospital it was noted that on the face the portion presents a distinct line of demarcation. The upper bo shown in the photograph (Plate IV), extends from the middle right eyebrow sloping upwards and then passes across the fore the left side, where it curves down again to a point just outs external canthus of the left eye. This border, with the exceptio small portion which has apparently healed, consists of a compa thin (8 mm. wide), inflammatory, red ulceration, which is superfic covered with a scanty scab. Along the right eyebrow the charac the lesion present a somewhat different aspect, showing a firm papi tous appearance. These papillomatous growths can be separate sometimes a minute quantity of pus can be squeezed out from b them. Thin scabs are found partially covering these lesions.

The right border of the facial patch extends from the right e horizontally outwards to a point within an inch of the ear; it the ceeds downwards and forwards towards the right angle of the mout stops abruptly within 1.5 cm. of this point. The border of the then turns slightly upwards and inwards towards the ala of the This right border is about 2.5 cm. broad and presents, especially to the lower angle, a pronounced papillomatous character, being dr scabby; where it has encroached on the region of the beard it is p by hairs. The largest papillæ, which are all closely aggregated tog are about the size of large pin-heads; from between these also sometimes a small quantity of pus can be squeezed out. The other portion of this border is covered with a thin scab, on removal of which a superficial ulcerative patch is exposed. The continuation of the lower border extends across the nose to within an inch of the tip as a superficial ulcerative edge.

From the left ala the lesion passes downwards to the left angle of the mouth, whence it extends still further down to the border of the jaw, along which it continues upward to the point of its first commencement. This area also is about 2.5 cm. broad, and again at the lower angle, *i. e.* between the mouth and the border of the jaw, the papillomatous character is well marked and numerous hairs protrude. These enlarged papillæ are raised nearly 6 mm. above the level of the normal skin. The lesion along the lower jaw is much narrower and the ulceration is quite superficial.

The whole area enclosed by this irregular border consists of one continuous atrophic scar which is whiter and thinner than normal skin. The eyelids of both eyes are practically destroyed, so that the patient cannot cover the eyeballs. Around the left eye the patch presents a raw, red, moist, easily bleeding surface, which extends for nearly 3 cm. downward from the lower border of the eye. The right eyelids present similar appearances except that the lower patch is not so extensive. The tears are continually running over these patches, particularly on the left side. The patient says that the disease is not even now particularly painful.

On the back of the right hand is an atrophic thin whitish scar which extends over the whole surface of the dorsum. The scar on the right side of the scrotum is about 3.5 cm. in diameter, is reddish and somewhat contracted, whereas the scar on the left thigh is smooth, thin, very white and not contracted. The lesion which was situated on the back of the neck has left a hypertrophic scar of distinctly keloidal appearance.

There are no enlarged glands. The patient has no cough; the bowels are regular, tongue fairly clean; he says that his general health has always been good. There is no history of syphilis, although the patient's wife had a miscarriage at four months, but no children. The wife's menstrual courses are very irregular. No tuberculous history in the family could be obtained.

Our first impression was that we had to deal with a case of lupus vulgaris, which would, therefore, have made its first appearance at the age of twenty-two years; but the primary lesion of all the patches presented the same feature, appearing first as a pimple and pustule, followed later by a superficial ulceration spreading, i jority of the lesions, peripherally. As the disease extended and in one lesion hypertrophic scarring had resulted. Thei history of the formation of lupus-nodules nor could any such found. The papillomatous form of the disease was in man suggestive of lupus papillomatosus. Tuberculosis cutis thought of and particularly tuberculosis verrucosa cutis (F Paltauf); but this disease is usually due to frequent contact composing animal matter and our patient had not been e such influences, although clinically various points of resembl sented themselves between this disease and our case. Tu verrucosa cutis begins as a papule, which later becomes pust after forming a scab, which falls off, takes on a papillomatous It spreads very slowly peripherally, and on lateral pressure a can be squeezed out from between the papillæ; but the han arms and feet are the only regions of the body where this di been found. The history of the lesions in our case, and the they healed spontaneously in three situations, would appear to cally almost sufficient to exclude tuberculosis of the skin in an usual forms. Scrofuloderma was also considered, but was on the grounds that the lesions in our case practically present of the typical features of this disease, nor were there any glands or constitutional symptoms to support this view.

For purposes of diagnosis three portions were excised, one f right eyebrow (inner border), one from the right border (pa tous) of the patch, and the third from the lower end of the lef near the chin. One portion was dropped into 5 per cent. f solution, and the other two into 95 per cent. alcohol.

PATHOLOGICAL HISTOLOGY.

Unstained sections treated with ordinary liquor potassæ sho presence of numbers of curious bodies which were doubly contou very refractive, whereas the tissue assumed a blurred appearance

The stained specimens showed a hypertrophy of the epidermi V, Fig. 1), throughout which numerous well defined miliary a of various sizes were scattered. Fig. 1, Plate VIII, represents one of the smaller miliary abscesses in the epidermis and contains two parasites. The epidermis was elsewhere considerably infiltrated with polynuclear leucocytes, and irregular masses of detritus covered certain portions of the epidermis. Large collections of granulation cells were massed in the corium, and numerous miliary abscesses (Plate V, Fig. 1, *b*) were also observed in the upper part of this region. A considerable amount of inflammatory material was also distributed throughout the corium, and in a number of sections there was distinct evidence of the formation of tubercle-like nodules (Plate V, Fig. 1, *n*) in the deeper portion of the corium.

Situated in all the miliary abscesses, and also among the granulation cells in the corium, as well as in the pseudo-tubercles, were numbers of doubly contoured, refractive, round and ovoid bodies (Fig. 1, *p*), varying in size from 10 to 20μ in diameter. Many of these forms presented buds of various sizes, and a vacuole was often, although not always, discernible. The bodies were usually found singly or in pairs, but they were sometimes arranged in groups. They were more numerous in the miliary abscesses than elsewhere. The contents of the bodies consisted of finely and coarsely granular protoplasm which took up the ordinary stains. Unna's method of staining for mastcells with polychrome methylene blue was employed on some sections and the coarse granules were found to be stained red, whereas the remaining portion of the protoplasm was practically decolorized. This reaction seemed to show that the coarse granules were basophilic.

Mastcells were somewhat numerous, but comparatively few giant cells (Fig. 1, *G*) were scattered throughout the corium; some of the latter enclosed one or more parasites. The contents of all the miliary abscesses (Plate V, Fig. 1, *a*, and Plate VIII, Fig. 1) consisted principally of large numbers of polynuclear leucocytes and nuclear fragments besides the parasites (*p*). Detached epithelial cells were sometimes present in the epidermal miliary abscesses.

Fig. 1, Plate V, represents a section from the cutaneous lesion; in it are seen numerous miliary abscesses (*a*) scattered throughout the hypertrophied epidermis (*e*) and corium (*c*). The section also shows the communication of one of the abscesses with the surface (*s*). Practically the entire epidermis was infiltrated with polynuclear leucocytes. The whole upper half of the corium (*c*) was the seat of acute and chronic inflammatory changes. Two hair follicles (*h*) were seen in the section, but they were essentially normal. The sudoriparous glands were also unaffected, and there did not appear to be any particular relationship between the diseased condition and the appendages of the skin.

Two pseudo-tubercles (*n*) were seen in this section in the lowe of the corium; these showed at their centres one or more parasit pseudo-tubercle was thus made up of one or more organism centre, surrounded by a number of polynuclear leucocytes, arou were three, four or more layers of epithelioid cells, while a larg of mononuclear or lymphoid cells formed the peripheral porti pseudo-tubercles were more numerous in the papillomatous or variety of the lesions; whereas in the acute or ulcerative form abscesses, and consequently more parasites, were present.

In the papillomatous lesions the papillæ were markedly hyper and the mouths of the hair follicles, especially from the regio beard, showed pronounced hyperkeratosis (*k*). The epidermis i not much altered and did not present the miliary abscess-for The corium consisted chiefly of large masses of granulation c very few of the miliary abscesses situated near the upper portion sites were found in all the abscesses. One could also find now a an organism among the granulation cells, but here they were n numerous; giant cells were present in only small numbers.

Generally speaking every miliary abscess contained one or mo sites (*p*), which were often in a budding stage. No parasites wer enclosed in cells except in a few giant cells which were situate deeper portion of the corium.

The organisms were first found and studied with the o hæmatoxylin and eosin stain. Under ordinary circumstanc could always be found in the tissues, but if the sections wer thick one could easily pass them over. They took the hæma stain just about as readily as the cell nuclei, but were more decolorized. We found that the most practical method of dem ing the presence of these organisms in the tissues was to imme section in a mixture of equal parts of liquor potassæ and gly when they were at once easily recognized. When we used Unna' chrome methylene blue stain the organisms were very prettily s the capsule and protoplasm being colored blue, whereas the granules were stained red. Methylene blue (aqueous) and eos gave good results. With carbolfuchsin and methylene blue th sule was sometimes stained red and the protoplasm blue.

The organisms were usually round or oval, and their resembla the parasites found in Gilchrist's case of blastomycetic dern already cited, was very close, although in the latter they were more numerous. The mode of development by budding could be followed out in the sections. In some instances the capsule had given way and the endosporium protruded as a bud (Fig. 12, Plate V), but more often the bud was formed by a pushing out of both endo- and exosporium (Fig. 11, *b*). The bud then grew larger and larger (Plate V, Figs. 3-13) until it reached almost the size of the parent, when the endosporium or protoplasm became divided by a clear space (*s*, Fig. 5). This stage was followed by a folding in of the capsule (*c*, Fig. 7) until the folds met and a separate organism was formed (Fig. 10). Sometimes a second bud was produced before the first had become detached (Figs. 11, 12, 13). A vacuole (see Figs. 2, 4, 6) was more often than not seen in the parasite, but there was much variation both in its size and shape. A few organisms presented rather a curious appearance (see Fig. 8); the section was stained with hæmatoxylin and eosin, but this organism did not stain, and presented, especially its central portion, a very refractive appearance, as if it had undergone some form of degeneration; surrounding the capsule was a delicate, fibrous-like structure of irregular outline. One shown in Fig. 9 also presented a similar appearance as far as the external, fibrous-looking, irregularly shaped coat was concerned, but the central portion stained in the same manner as in the other organisms. Fig. 15 shows also an unusual form where the protoplasm has contracted into a half-moon shape. Sometimes a number of parasites are collected into a group (Fig. 16), which is usually situated in the corium and rarely in an abscess. Plate VIII, Figs. 3, 4, and 5, show varieties of organisms enclosed in giant cells. In Fig. 5, Plate VIII, the vacuole, both in the parent and in the bud, was particularly clear and well marked. Four parasites were found enclosed in a giant cell as shown in Fig. 4; three of them were in various stages of gemmation.

CULTURES.

After careful sterilization of the surface of the papillomatous lesions, cultures on plain agar were taken from two places from the pus, which after pressure oozed out from between the growths. Other similar cultures were taken from the diseased area after removal of the scab, and from one excised portion of the tissue.

Although every possible precaution was taken in order to avoid any contamination, yet the cultures from the tissues contained a profuse growth of pus organisms, and in only one instance a single large colony of the parasites developed. These cocci were not further noticed, as they were not found in the pus and could not have caused the chronic process.

Both of the tubes inoculated with the pus showed in one week a pure growth of the organism about to be described.

Morphology.—This was first observed in specimens taken from young colonies from the surface of plain agar, and mounted in salt solution. They may be studied either with the No. 7 Leitz objective or by means of the oil-immersion lens.

The most usual form consists of a large, round, oval, or irregular, highly refractive body, surrounded by a doubly contoured membrane, consisting of an exosporium and an endosporium (Plate VI, Figs. 5, 13; Plate VII, Fig. 1). These bodies contained many refractive granules resembling fat granules, which at times show Brunonian movements, and a number of finer dark granulations. At times one or more large vacuoles can be seen within the protoplasm.

These bodies may be stained while fresh by adding a small amount of an aniline dye to the salt solution. If the precaution be taken to tease out carefully a dry bit of a colony on a cover-slip or slide, and the specimen be then diluted with a drop of salt solution and dried in the air or high above the flame, the organisms can be stained as are ordinary bacteria.

Treated thus with Loeffler's methylene blue or carbolfuchsin, the membrane stains deeply while the protoplasm takes on a lighter stain. The large pseudo-fat granules do not retain the dye, while, on the other hand, the small granulations stain more intensely than any other portion of the cell. At times the finely granular protoplasm does not completely fill the cell, but is separated from the membrane by a clear faintly staining hyaline area. These bodies remain unchanged in pure glacial acetic acid, and in 25 and 50 per cent. hydrochloric acid, but in pure hydrochloric acid they disappear.

Attempts were made to ascertain the chemical nature of the large granules, but they gave no reaction when treated with iodine, and hot

Millon's reagent and ether, although applied twelve times in succession, failed to dissolve them. The membrane also failed to give the cellulose reaction, but the fine black granulations would appear to be albuminous, since on boiling fresh specimens with Millon's reagent they often appear in the protoplasm as brownish-red particles.

The first colonies which were obtained from the original pus cultures consisted almost entirely of budding forms of the parasite (Plate VI, Figs. 4, 5, 6, 7, 17, 18, 19).

Mode of Growth.—The development of the parasite was then studied by means of small particles of the growth introduced into a hanging drop culture of gelatine and then allowed to grow (Plate VI).

From the round or oval bodies there occurs a growth of many long branching threads like mycelium, which are divided into shorter hyphæ by intervening fine transverse lines (Plate VI, Fig. 12). The protoplasm of the hyphæ is at first clear (Plate VI, Figs. 17, 18), but later it becomes beset with numerous fine granules or larger refractive bodies resembling oil drops (Plate VI, Fig. 3). In old cultures the protoplasm seems to arrange itself into long or oval hyaline bodies separated one from the other by a clear interval.

The numerous drawings of Plate VI represent many of the varieties which were found in the hanging drop cultures.

After several days, at various points along the sides of the hyphæ a number of knob-like projections (Plate VI, Fig. 15) of the limiting membrane may be seen, which gradually enlarge and at last form full-grown, round cells, or conidia, joined to the mycelium by means of their stems or sterigmata (Figs. 14, 15). These conidia may possess a double contoured membrane, together with fine or coarse granules. They may also become detached from the hyphæ, when they can either repeat the cycle of development just described, or increase by the process of budding and the formation of daughter cells. This latter phenomenon is of interest, since it is the only evidence of increase to be found in the tissues. The growth of the cultures is always accompanied by the formation of envelope and small crystals of oxalate of lime (Plate VI, Fig. 22).

Occasionally, in about the third generation of various cultures, certain colonies appeared to consist chiefly of the budding variety, only short hyphæ being observed here and there (Plate VI, Figs. 17, 18). In old cultures there appeared at times a mycelium which was finer in structure than the usual form observed.

Appearance of Cultures.—The organisms grow best on glycerine agar and potato, and develop slowly. In about seven days numerous fine grayish-white colonies appear on the surface, which later become pure white and show minute prickles, thus resembling a number of min chestnut burrs scattered over the medium. They can attain t a split pea; they grow about two millimetres above the surfac numerous, form a confluent membrane, suggesting a portion of of a white mouse stretched over the surface of the mediı growth is always firmly adherent to its substratum, and portio latter material are often removed with the colonies.

After the cultures have been passed through several gener animals the loss of the mycelium causes these masses to appear flat gelatinous drops spread over the surface of the medium. fluid cultures, for example, with bouillon, the parasite forms interlacing meshwork through the medium, and no individual b be found in the culture fluid.

In plate agar cultures the colonies show under a low magnifyi a granular centre from whose periphery fine ramifying branches g

Colonies also develop readily on plain nutrient gelatine, 20 beer-wort gelatine, plain agar, 5 per cent. beer-wort agar, bouill beer-wort (unfermented beer), Dunham's solution, milk, agar ma infusions of the various organs, Loeffler's blood-serum mixtu human blood serum. Milk is not coagulated nor acidulated; forms in Dunham's solution, and gelatine is not liquefied. No a fermentation occurred, nor was the formation of any gas observ several weeks growth in the fermentation tubes containing gluc tose and saccharose bouillon.

Although cultures have been left for several months on ap dried-up tubes, yet when transferred at the end of this time th luxuriantly, and in one case even proved pathogenic after this le time.

The parasite will not grow as a strict anaerobe. This was pr making several stab cultures into glycerine agar and covering t face with melted gelatine. This tube was then placed in a Buch with pyrogallic acid and KOH, and allowed to remain for one m room temperature. At the end of this time the bodies intr showed no evidence of increase.

The organisms develop in about one month when subjected to perature but slightly above freezing, and grow most favorably at perature ranging from 18-36° C. Their maximum temperatu growth is 40° C.

INOCULATION OF ANIMALS.

Fresh bits of tissue were introduced subcutaneously into a mouse and a guinea-pig. The mouse died in 24 hours of a pn coccus septicæmia. The guinea-pig showed no change although observed for several months. Another guinea-pig was inoculated in the peritoneal cavity with diseased tissue after a laparotomy. After two months the animal was killed, but showed no pathological changes, with the exception of a few necrotic areas in the liver. Cultures, cover-slips and hardened specimens failed to reveal the presence of any parasites or bacteria in the liver, and cultures from the heart's blood and other viscera were negative.

These inoculations are only of negative importance, as they enable us, in conjunction with the facts mentioned above, to exclude tuberculosis. A number of inoculations were made also with pure cultures of the organism. Four white mice were inoculated at the root of the tail. They survived the inoculation one month and were killed at the end of this time; the autopsy and cultures were negative. Two guinea-pigs inoculated subcutaneously with teased bits of cultures failed to show any reaction during two months, and several similar animals injected hypodermically with teased particles in bouillon gave negative results.

Several attempts were also made to reproduce the disease by implanting pure cultures upon scarified areas, or introducing them into small subcutaneous pockets made in the skin of a dog. These inoculations resulted only in abscesses containing the ordinary pus organisms. The organism was also introduced into the circulation of a rabbit, but when the animal was killed, two months later, the autopsy showed nothing remarkable, and stained hardened specimens of the lung showed these to be normal.

The following inoculation experiments, however, proved successful:

Exp. 1. Dog 1. The neck of a small dog was shaved and cleaned; the external jugular vein was exposed by Dr. Flexner, and one cubic centimetre of a suspension of teased colonies from a growth from the original pus was injected in the direction of the circulation. During the next two months the animal became somewhat emaciated and developed a discharge from the nose; at the end of that time it was killed.

Autopsy. Left external jugular vein obliterated and changed to a fibrous cord.

Lungs. Upon opening the thoracic cavity, both lungs presented a striking picture. Projecting from the entire pleural surfaces of both lungs there were numerous, generally discrete, pea-sized or larger, firm, light yellow nodules, which on section extend for the distance of from 0.5 to 2 centimetres into the lung substance. These areas are yellow, but are dotted here and there with lighter grayish-yellow apparently softer spots, half a pin-head in size. These nodules are round, their peripheries are sharply marked off from the surrounding healthy lung tissue, and they are of about the consistence of a fresh pea. On section of both lungs numerous similar bodies were found scattered over the surface, but no cavities or caseous areas were seen. The bronchial glands were enlarged, firm, light yellow on section. The other viscera presented nothing of interest.

Bits of the pulmonary nodules and bronchial glands were teased finely and mounted in equal parts of 5 per cent. KOH and glycerine. A large number of the organisms were apparent in both specimens as highly refractive bodies with a well marked doubly contoured membrane; numerous budding varieties were also found. Fresh sections were placed in 10 per cent. formalin, followed by alcohol, and then stained with Stirling's gentian violet solution and decolorized with acetic acid 1-1000 and oil of cloves (Flexner's method). The nodules were then seen to consist of large central areas of necrosis containing an occasional giant cell and many fat drops, and surrounded by a zone of large epithelioid and lymphoid cells. The parasites are for the most part scattered throughout the necrotic area. The membrane stains dark blue, and the protoplasm a lighter blue, but no further differentiation can be made out. From the study of these bodies in fresh sections it is quite evident that there has been an extraordinary increase of them within the lungs, as from 6 to 10 spores often form the nucleus of an entire colony, and not a great number of these colonies were originally injected. No mycelium was present in the tissues.

Cultures.—The surface of the lung nodules was sterilized and relatively large portions were then dissected out with sterile instruments and placed on the surface of tubes of human blood-serum, glycerine agar, and beer-wort agar. In one week typical and pure growths occurred from the edges of the portions of tissue introduced, which consisted of bodies morphologically identical with those described above, and which later formed a profuse mycelium as in the cultures from the original case.

Pathological Histology. The smaller nodules scattered through the lung tissue consist of large accumulations of round, oval or elongated epithelioid cells and small round lymphoid cells, together with a moderate number of polynuclear leucocytes. These latter often accumulate around or apparently even invade the interior of some of the parasites which are probably dead. The larger and older nodules have undergone coagulative necrosis and in places contain groups of polynuclear leucocytes. Nodules stained for fibrin and glycogen gave negative results. Sections of pieces hardened in Flemming's solution show numerous large or small black fat drops for the most part within the caseous areas, but at times they are seen in younger nodules, or in large *Staubzellen* on the edges of the diseased tissue. The younger nodules contain a few giant cells which often include a parasite.

The bronchial glands consist of a mass of confluent pseudo-tubercles intermingled with smaller areas of normal lymphoid tissue. These tubercles contain a number of giant cells, leucocytes and parasites, but show very little necrosis.

Both in the connective tissue of the larger bronchi and in the thick fibrous trabeculæ of the bronchial glands there are a number of mast-cells present.

Exp. 2. Dog 2. The next animal inoculation was made by injecting about two cubic centimetres of a suspension in sterile bouillon made from portions of the teased nodules of the lung of Dog 1 into the external jugular vein of a second dog.

In about two weeks the animal developed a sniffling cough and showed a well marked secretion of pus from the nose. This condition lasted several weeks, but, although the pus was examined microscopically several times, only one body having some resemblance to the parasite was found.

The animal was killed at the end of two months, and the autopsy showed each lung to contain about twelve firm, grayish-yellow, sharply circumscribed, round nodules, varying in size from a pea to a sparrow's egg, with an average diameter of from one to two centimetres. They are apparent as slight elevations over the surface of the pleuræ, and also as circumscribed nodules throughout the lung tissue.

The gross appearance of these nodules in the lungs of both dogs much resembles that of metastatic carcinoma. Nothing else of interest was noticed macroscopically.

The lung nodules from Dog 2, when teased and mounted in salt solution, showed a number of round or oval doubly contoured bodies exactly resembling those obtained from the lung of Dog. 1. The bronchial glands contained no organisms.

Pure cultures of the blastomyces were obtained by spreading large portions of the lung nodules over the surface of beer-wort agar.

On microscopic examination these nodules did not show such marked necrotic areas as were observed in the lungs of the first dog, but they here and there exhibited a tubercular formation with giant cells, epithelioid cells and lymphoid cells. It was not an uncommon thing to find a single adult organism apparently situated in the centre of an alveolus, which was filled up with a large collection of epithelioid cells, with a number of polynuclear leucocytes directly surrounding the parasites (Fig. 2, Plate VIII). This picture shows very closely what was sometimes seen in the tissues of the original case. The general appearances were those of a chronic inflammation, and one would not be likely to regard the lesions as those of a neoplasm. Organisms of the budding form, without mycelium, were found in all the nodules, but not so many apparently dead forms were seen as in the first dog's lung. Giant cells were not numerous; most of those seen contained one or more parasites.

At the periphery of the younger nodules the alveoli showed marked swelling and projection of the epithelial cells, and often in the centre of an alveolus completely filled with epithelial cells one or more of the parasites could be made out. It would appear that this proliferation forms the beginning of the pathological process.

Exp. 3. A guinea-pig was inoculated with a suspension made from the lung nodule from Dog 2 into the peritoneal cavity, but an autopsy two months later showed no pathological lesions.

Exp. 4. Dog 3. A third dog received also an intravenous injection of about two cubic centimetres of a bouillon suspension from the lung of Dog 2, and at the autopsy two months later the lungs were seen to contain a moderate number of small, grayish pseudo-tubercles, the size of a pin-head. These nodules when teased in salt solution were seen to contain a few of the parasites and many fatty endothelial cells. The lesions were less marked and fewer than in the two previous dogs, showing that the organism was apparently losing its virulence.

Exp. 5. A guinea-pig was inoculated in the peritoneal cavity with some finely teased nodules from the lung of Dog 1. In about two weeks the epididymis on both sides became tense, swollen and painful, and later these swellings opened upon the skin surface and discharged a large amount of pus. This, when examined under the microscope, was found to contain a number of large doubly contoured bodies, many of which possessed small budding knobs and daughter cells. The formation of these knobs was also beautifully shown, and consisted in the rupture of the thick, doubly contoured membrane, and the protrusion through this opening of the protoplasm of the cell surrounded by the thin endosporium. These knobs later became invested by an outer membrane, and daughter cells were thus formed. Cover-slips stained for pus cocci gave negative results. The cultures were not contaminated.

These abscesses gradually healed, and at the autopsy, one month after inoculation, nothing was found except the fibrous scars of the pus cavities.

Exp. 6. Sheep. The external jugular vein of a sheep was exposed and 2 cc. of a suspension in bouillon of several fine colonies from a pure culture of the organism derived from Dog 1 were injected into the vein by a sterilized hypodermic syringe. The needle was simply withdrawn and no ligatures were used.

From time to time a thin purulent secretion from the nostrils was noticed, but no parasites were found in the pus. In about five weeks the animal was killed, and both lungs contained a number of gray, semi-translucent, firm nodules, varying in size from a pin-head to a split pea. These nodules contained numbers of doubly contoured bodies, and a pure culture of the organism was obtained on beer-wort agar. Surrounding the nodules was quite a marked belt of young fibrous tissue.

Exp. 7. Horse. Many fine colonies from beer-wort agar culture from the lung nodules of Dog 1 were also teased and suspended in bouillon, and 2 cc. of this fluid were injected into the right external jugular vein of a horse, while at the same time a similar quantity was introduced into the subcutaneous tissues of the neck near the seat of the former injection.

In about thirty days there appeared an indurated abscess, the size of an egg, which soon opened superficially, and discharged a thick, creamy, yellow pus. This abscess was situated about midway between the angle of the jaw and the shoulder, on the right side of the neck, just beneath the seat of local inoculation. The skin over this abscess was shaved and sterilized, the abscess was thoroughly opened, and about 10 cc. of pus was collected in a sterile Petri dish. The opening closed in one week.

Cover-slips from this pus showed that it contained large numbers of doubly contoured refractive parasites, together with many eosinophiles, while cultures on beer-wort agar in one week showed a number of elevated gray, irregular membrane-like colonies. These consisted of doubly contoured blastomycetes, developing hyphæ as in cultures previously described. Plate VII represents a number of the varieties which were found in a teased colony and their method of growth. The tendency of the organism to develop by budding was again more pronounced at first, as in the cultures from the pus obtained from the original case. Although several cultures were made, none of the ordinary pus organisms developed, and cover-slips from pus also failed to reveal any such organisms.

At the end of two months the horse was killed and, although the superficial opening of the abscess had healed, yet in the deeper tissues of the neck a thick fibrous capsule was found surrounding a mass of thick yellow pus, which contained numbers of the blastomycetes.

On opening the thorax the pleural surfaces of both lungs showed a moderate number of firm, yellowish, sharply circumscribed nodules, varying in size from a split pea to an almond. Some nodules contained a central pus cavity, while from others pus could be squeezed as from a sponge. These nodules were seen throughout the cut surface of both lungs. Neither the bronchial nor the cervical glands were affected, and the rest of the organs were normal.

The cultures from the pulmonary nodules became contaminated, but cover-slips from teased nodules contained typical blastomycetes.

Exp. 8. A teased nodule was put into the peritoneal cavity of a guinea-pig and the animal was killed in two months. Just beneath the point at which the tissue was introduced, between a loop of intestine posteriorly and the abdominal wall anteriorly, there were many organisms similar to those described above. It was impossible to take any cultures.

Exp. 9. A young pig (not guinea-pig) and several mice were inoculated subcutaneously with the pus from the abscess of the horse, but with negative results.

Exp. 10. A number of teased colonies from a beer-wort agar culture from the pus from the horse's neck were introduced into the peritoneal cavity of a guinea-pig, and in two months the animal was killed. On opening the abdomen a pea-sized, ovoid, yellow nodule was found enclosed within folds of peritoneum and attached to the testicle by a fibrous, long, thin pedicle. This mass consisted of a thin capsule, including a mass of soft semi-caseous pus, which contained numerous doubly contoured, refractive bodies. Cultures from the pus on blood-serum showed a pure growth of the parasite.

In addition, an attempt was made to produce a lesion in the liver of a guinea-pig by injecting a teased culture suspended in bouillon from the lung of Dog 1 into the mesenteric vein. At the autopsy about eight weeks later the liver was found to be normal, but at the point of entrance of the hypodermic needle a small abscess had developed. This was hardened in alcohol, and when stained was seen to consist of numerous pus cells and a large number of parasites, including many budding forms. Although the pus from the horse's neck was injected subcutaneously into one guinea-pig, and introduced into the peritoneal cavity of a second animal, the results in both cases proved negative.

Speaking generally, the effects produced on animals were the formation of typical pseudo-tubercles with caseation in the lungs of the first dog; in the second dog the necrosis was not so pronounced, although there was some slight caseation, and in the third dog the lesions were less marked and presented more the characters of foci of chronic inflammation. In the horse a local abscess was formed in the neck at the seat of inoculation and pseudo-tubercular nodules were formed in both lungs. In the sheep the pulmonary lesions were also of a pseudotubercular character. In the three guinea-pigs which were successfully inoculated, encysted abscesses were the result, and a much larger number of organisms were contained in them than were injected.

The parasitic organisms in all the lesions presented the same general features and appeared identical in character with those in the patient. No mycelium was ever found in any of the lesions, although such developed in the cultures.

Many staining methods were tried on the diseased tissues from the animals, but the organism could be distinguished and studied well with the ordinary hæmatoxylin and eosin stain if the sections were thin.

If overstained with the hæmatoxylin the parasites would take up this stain, but if the eosin was used in excess they assumed the latter stain by preference. With the carbolfuchsin and aqueous methylene blue, the organism, particularly in the nodules from the lungs, could be made to acquire a double stain, the capsule being red and the protoplasm blue. The same effect could also be produced with eosin and aqueous methylene blue. Although safranin was tried and the organisms took up the stain well, they were less easily found among the surrounding stained cells. Flexner's method of staining with Stirling's gentian violet and decolorizing with acetic acid caused the parasite to stand out very prominently, but did not differentiate the contents of the organism.

CLASSIFICATION.

In our preliminary communication we classed our organism as an oidium for the following reasons: the organism did not ferment glucose, saccharose or lactose, and, although it only developed by gemmation in all the tissues, human and animal, yet upon artificial culture media it developed mycelia with the formation of conidia. After consulting Brefeld's writings, and also after a personal conference with Dr. Erwin Smith of Washington, an acknowledged authority in mycology, we were inclined to consider the parasite as an oidium. On further investigation we find that mycologists are not at all certain about the classification of either the yeast fungi or the oidia. We find that there are a number of wild yeasts which do not ferment sugar and which form mycelium on the usual bacteriological media. In accordance with the prevailing custom in the nomenclature of organisms presenting characters similar to ours, we now prefer to designate the parasite as a blastomyces rather than an oidium.

COMPARISON OF OUR ORGANISM WITH THOSE OF PREVIOUS OBSERVERS.

In Busse's case the primary lesion was thought at first to be a sarcoma and the internal lesions resembled those of chronic pyæmia. Gilchrist's case resembled chronic scrofuloderma of the skin, wherein it resembled one variety of the cutaneous lesions of the present case. Corselli and Frisco described their case as one of sarcoma of the mesenteric glands, and Curtis speaks of his case as a tumor of myxomatous appearance of the thigh and loin.

The lesions in our patient differ then from those in all the previous cases with the exception of Gilchrist's case of blastomycetic dermatitis, in that ours simulated lupus vulgaris, and in the inoculated animals presented lesions somewhat resembling tuberculosis with acute and chronic inflammation. Our organism was also distinctly pyogenic both in the original case and in inoculated animals.

The organism in our case, although apparently differing but little from those described in previous human cases, is markedly distinct in its method of growth on media by profuse mycelium and conidia. In all the other cases the growth has been described as being chiefly by gemmation. In her experimental work on blastomycetes Rabinowitsch demonstrated that a number of wild yeasts which were pathogenic in animals did not ferment sugar and formed mycelium. Whether all these parasites have the power of producing different pathogenic pictures according to the organism which they attack, or whether they are varieties of the same species, is a question which is very difficult of solution in the present crude state of our knowledge regarding this class of micro-organisms.

COMPARISON OF OUR ORGANISM WITH THE SO-CALLED CANCER PARASITES.

As we have already mentioned, many of the investigators who have reported cases where blastomycetes were believed to be the cause of pathogenic lesions, have made the assertion that the bodies present in cancerous tissues, which some previous observers had wrongly described as protozoan parasites, are really blastomycetes. Not a few authors have also described many of the lesions which have been produced in animals by inoculation with blastomycetes, as genuine neoplasms, and have adduced these in support of the parasitic origin of malignant tumors. Sanfelice, especially, described the lesions produced in the mammary glands of a bitch and in the comb of a cock as presenting a carcinomatous appearance. Sanfelice, Roncali, Kahane, Binaghi and others have also asserted that the curious bodies which are often found in sarcomatous and epitheliomatous tissues are really blastomycetes, although they have failed to obtain them in cultures and have not succeeded with inoculation experiments. One of us (Gilchrist) has already made comparison between the blastomycetes which he found in his case and the so-called parasites in twenty-three cases of epithelioma of the skin, and demonstrated that there were very few points of resemblance between these forms. He concluded that the evidence appeared to be strongly against the idea that the bodies found in cancer are blastomycetes. We have compared our parasites in this case again with the so-called cancer parasites, and feel still more strongly in favor of the view that the bodies found in cancer are really the result of epithelial degeneration. The same arguments can be presented here against the view that the cancer bodies are blastomycetes, which one of us (Gilchrist *) advanced in regard to these bodies being considered as protozoa. These are mainly as follows: (1) The so-called cancer parasites found in twenty-three cases of cancer, five of which were of the lip, bear very little resemblance to the blastomycetes either of Gilchrist's case or the present one. (2) They are by no means present in every case of undoubted cancer. (3) They are often found in other diseases, *e. g.* in benign growths of the skin, in tuberculosis cutis, in syphilitic ulcers of the skin, and in other cases. (4) There is an entire absence of uniformity in the appearance of the cancer bodies, those described for one case being often entirely unlike those seen in another. (5) The descriptions given of developmental stages are vague and by no means convincing. (6) Special stains and refined technique are required to bring out many of the so-called cancer parasites, whereas the blastomycetes are demonstrable by all methods and stain readily. (7) The blastomycetic dermatitis of Gilchrist's case and the present one do not approach the clinical or pathological characters of epithelioma, but bear more resemblance to tuberculosis of the skin. (8) No positive results have ever been obtained from the numerous inoculation experiments which have been made. (Roncali has isolated what he calls a blastomyces from an epithelioma, but one can often cultivate many kinds of fungi from superficial epitheliomata.) (9) The ready demonstration of the blastomycetes by liquor potassæ does not apply to the so-called cancer parasites.

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* *Johns Hopkins Hospital Reports*, i.

The theory of the blastomycetic origin of malignant tumors has also received little support from experimentation. Shattock and Ballance made a large number of cultures from malignant tumors upon all of the various culture media, but with negative results. At the suggestion of Dr. Flexner we have also made cultures upon beer-wort agar (the most favorable medium for yeasts) from about ten sarcomata and carcinomata. Our method consisted in sterilizing by heat both sides of a small block of tumor immediately after removal, and then punching out a cylinder with a sterile trochar, teasing the tissue in a sterile Petri dish, and plating the finely divided tumor in beer-wort agar. The plates usually remained sterile, and although an occasional colony developed this never consisted of yeasts. The plates were observed for weeks. The theory of the blastomycetic origin of malignant tumors, we think, needs much more confirmatory evidence before it can be accepted.

TREATMENT.

We had intended to place the patient under the influence of ether and curette the cutaneous lesions very thoroughly, and then apply the nitrate of silver stick to the denuded surface. In view of the fact that the parasites were not very numerous and the course of the disease had shown itself to be exceedingly chronic, we considered we had good reasons for supposing that the disease could thus be easily cured, especially since the neighboring lymphatic glands were unaffected. But the patient was called away from the hospital on account of the serious illness of his wife, and he has remained without treatment up to the present time. We received a letter from him recently, six months after he left the hospital, in which he says that his face and eyelids have almost healed up. The disease, all through its course, has shown a strong inclination to spontaneous cure. The prognosis, in our opinion, therefore, is good.

SUMMARY.

The case reported in this article is one of a somewhat extensive cutaneous disease, which occurred in a man, 33 years of age, who gave the following history: The disease first made its appearance, eleven and a half years ago, at the back of the left ear, as a pimple which soon became pustular. The process extended forward very slowly and gradually encroached upon and covered almost the entire face, the central portion of which now presents an atrophic cicatricial condition. Another similar lesion occurred, one month after the primary invasion, on the back of the hand, which healed in about four years, after treatment with caustic. A third lesion appeared on the right side of the scrotum (six months after), which increased in size for a year and then healed spontaneously. A fourth inoculation appeared on the anterior surface of the left thigh just above the internal condyle, and grew for a year, after which it gradually healed spontaneously. A fifth lesion appeared on the back of the neck and also healed spontaneously after growing for a year. The disease when first examined presented many of the features of a lupus vulgaris.

There were no enlarged lymphatic glands and the patient's health had always been good. The family and personal history revealed no syphilitic or tuberculous taint.

Sections from the cutaneous lesions showed the presence of what appeared to be budding blastomycetes. The sections also presented pathological features similar to those seen in the first case recorded by Gilchrist; in many sections almost typical tubercles were found.

The organisms in the tissue are chiefly spherical, unicellular bodies varying from 10-20 μ in diameter, and consist of a doubly contoured membrane, which encloses a fine granular protoplasm with sometimes a vacuole. Many budding forms in various stages were found; no nucleus could be demonstrated, neither were any mycelium or hyphæ present in the tissues. The parasites were almost always found outside of cells, comparatively few being enclosed in giant cells.

Pure cultures of the organism were obtained directly from the cutaneous lesions in two places from the pus squeezed out from between the papillomatous variety of the lesion. The organism grew on all ordinary media, and especially well on potato and beer-wort agar. The cultures showed both budding forms and a fairly profuse mycelium. Older cultures, carried through many generations, produced sometimes little or no mycelium. The organisms in the cultures were round, ovoid, doubly contoured, refractive bodies, varying in size from about 10 to 20μ in diameter. The mycelium was, on rare occasions, of two varieties, very fine and also coarse with sessile buds and conidia.

Dogs, a horse, a sheep and guinea-pigs were successfully inoculated, the most striking results being nodules, grossly simulating tumors, in the lungs. Microscopically these nodules were of a chronic inflammatory nature and contained numerous parasites identical in appearance with those in the patient. In the tissues of none of the animals successfully inoculated was any mycelium found. Since our organism did not ferment sugar and produced in cultures mycelium, it may either belong to the blastomycetes or to the oidia, but in conformity with prevailing nomenclature we regard it as a blastomyces.

We shall term the disease which has been produced by this organism *Blastomycetic Dermatitis.* We give the name *Blastomyces dermatitidis* to the parasite which we have isolated and described in this paper.

In closing we are of the opinion that it would be advisable to examine more carefully all tuberculous lesions of the skin, and especially those of tuberculosis verrucosa cutis, for the presence of blastomycetes. This can be readily and rapidly done by soaking the unstained sections in ordinary liquor potassæ, when the organisms if present will stand out as doubly contoured refractive bodies.

DESCRIPTION OF PLATES IV—VIII.

PLATE IV.

Photograph of patient showing the cutaneous lesions of blastomycetic dermatitis.

PLATE V.

Fig. 1 represents a microscopic section of blastomycetic dermatitis. The epidermis (*e*) is hypertrophied and encloses numerous miliary abscesses (*a*), which contain the parasitic organisms (*p*). In the corium (*c*) miliary abscesses (*b*) are also present as well as pseudo-tubercles (*n*), which enclose parasites (*p*). The corium is filled with masses of granulation-tissue cells and contains a few scattered giant cells (*G*).

Figs. 2-16 represent various forms of the blastomycetic parasite as observed in the tissues of the patient. Various stages of the budding process are shown. The refractive double contours of the enveloping membrane and the protoplasmic contents of the parasite are depicted. The organisms drawn in Figs. 8 and 9 appeared to have an outer irregular fibrous coating. Fig. 16 shows a group of parasites in the corium.

PLATE VI.

Figs. 1-21 represent various growth-forms as observed in a colony of the blastomyces cultivated in a hanging drop of gelatine on the slide. Figs. 6, 7, 10, budding yeast forms. Figs. 16, 17, 18, beginning development of hyphæ. Other figures show various mycelial developmental forms with conidia. Fig. 22, crystal of calcium oxalate.

PLATE VII.

Figs. 1-16 represent a number of varieties of the developmental stages of the blastomyces as obtained from a colony from the pus produced in the horse's neck. Fig. 1 is a single round organism with a large clear space in the centre. Direct budding forms are seen in Figs. 2, 4, 14 and 16. A sessile bud on mycelium is shown in Fig. 8. Mycelial threads forming from the organism are seen in Fig. 3, whereas in others the mycelium shows attempts at forming the round or oval form directly in the axis.

PLATE VIII.

Fig. 1 shows a small miliary abscess situated in the epidermis. It contains two parasites, a number of polynuclear leucocytes, nuclear fragments, and nuclei of detached epithelial cells.

Fig. 2 represents a portion of one of the most recently formed nodules from the lung of the first dog inoculated. The organism is in the centre, a number of polynuclear leucocytes lie between the parasite and the epithelioid cells, and lymphoid cells constitute the peripheral portion.

Figs. 3, 4 and 5 show giant cells which were found in the deeper portion of the corium of the patient's cutaneous lesions. The vacuole in the parasite and the budding are well marked in Fig. 5.

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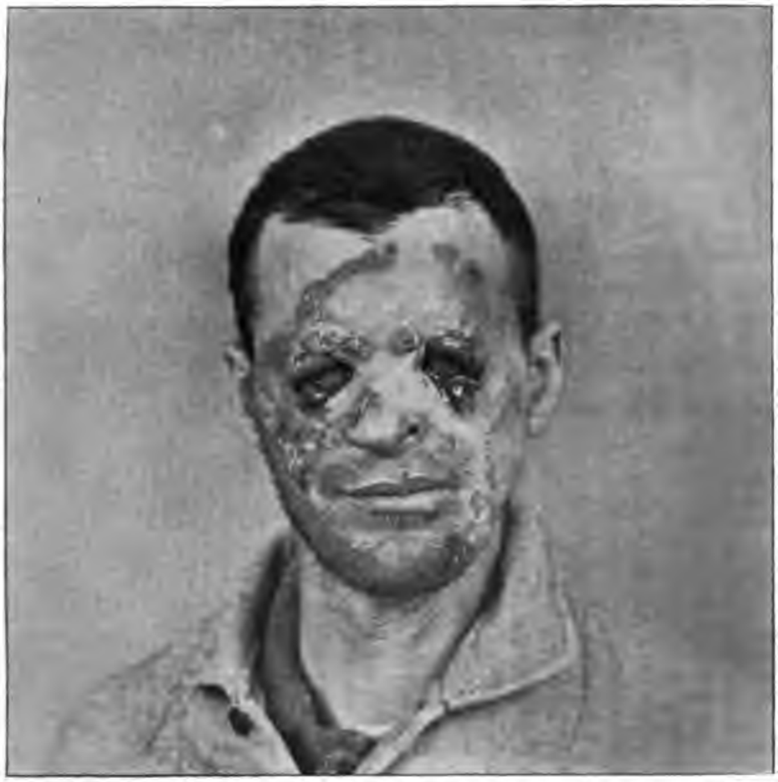
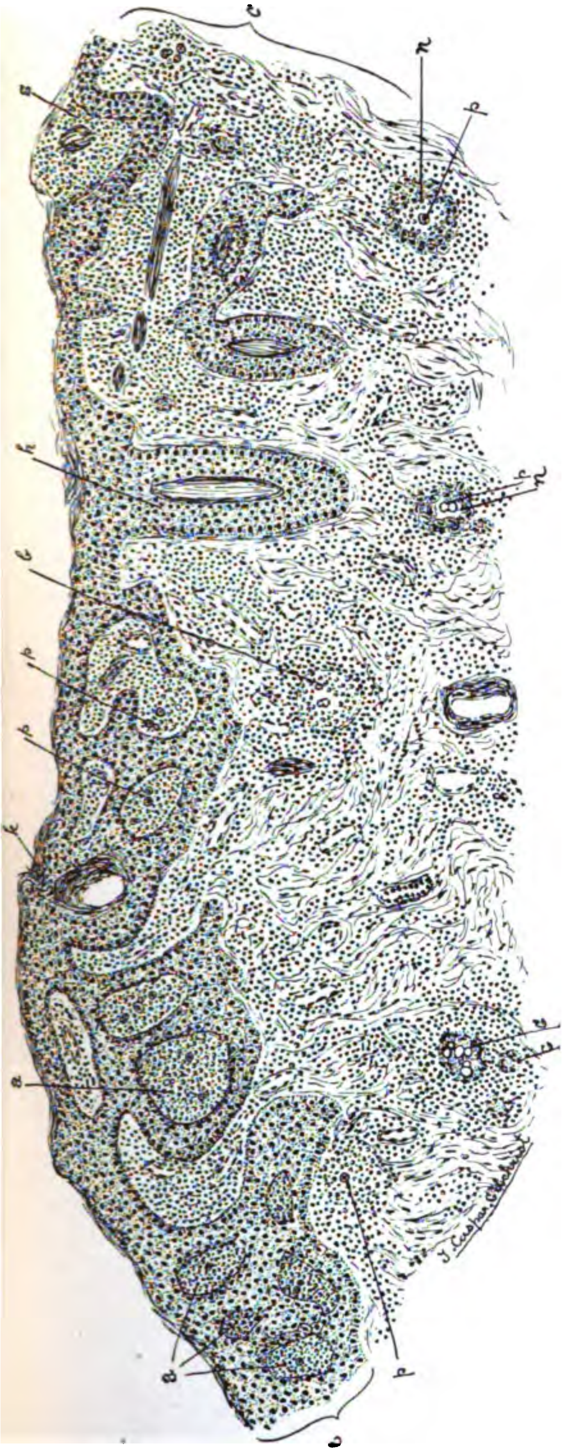


PLATE V.



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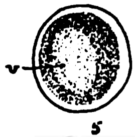

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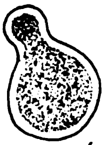

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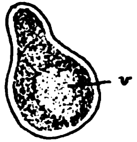

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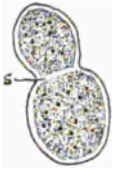

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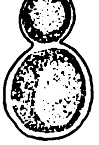

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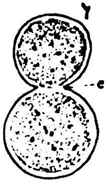

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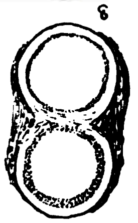

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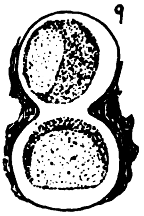

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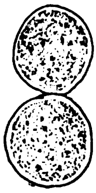

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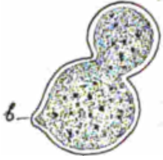

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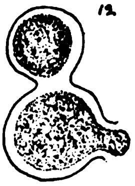

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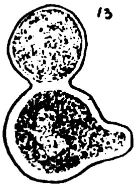

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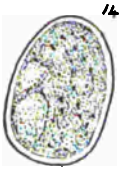

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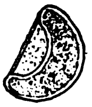

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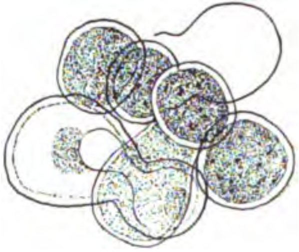

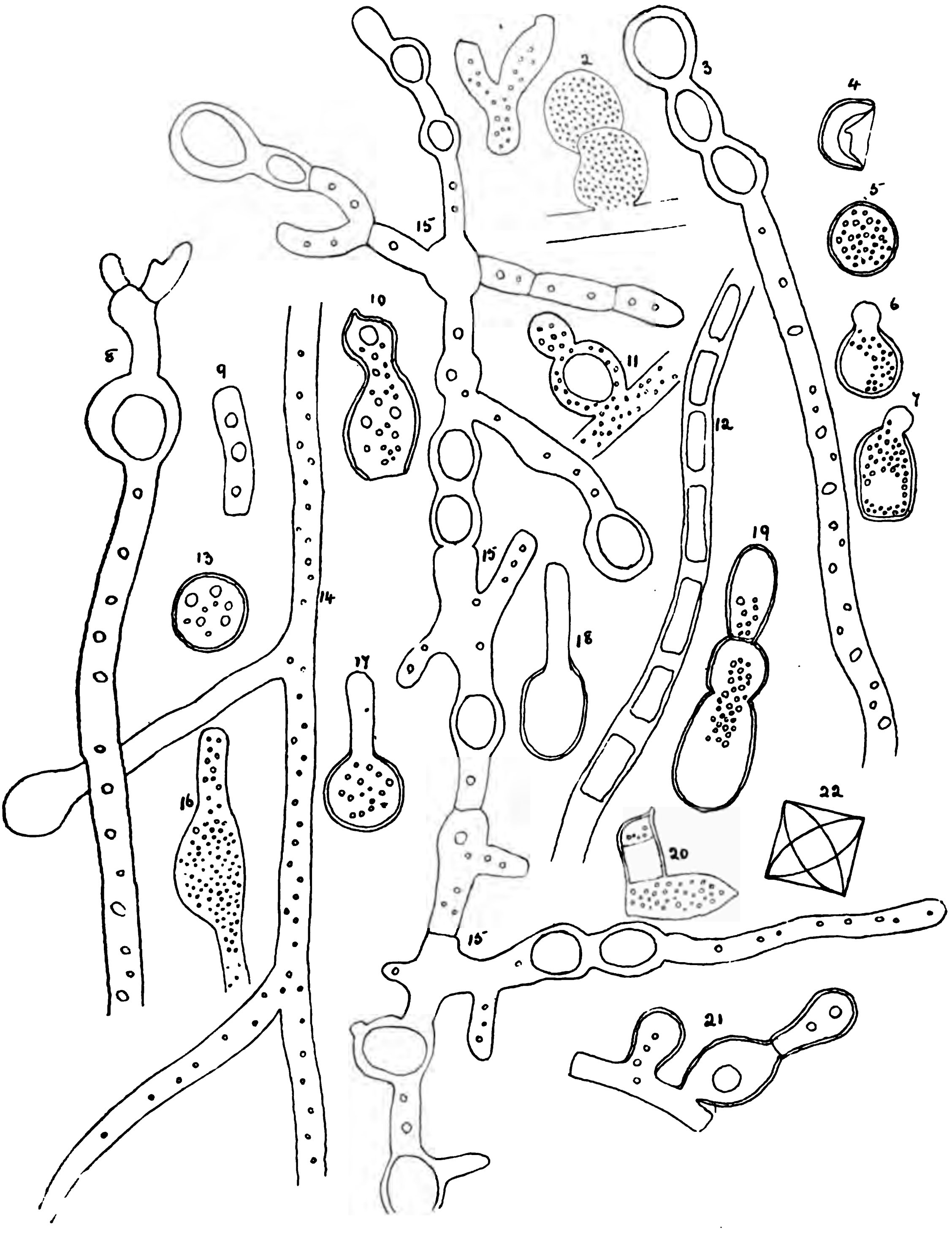
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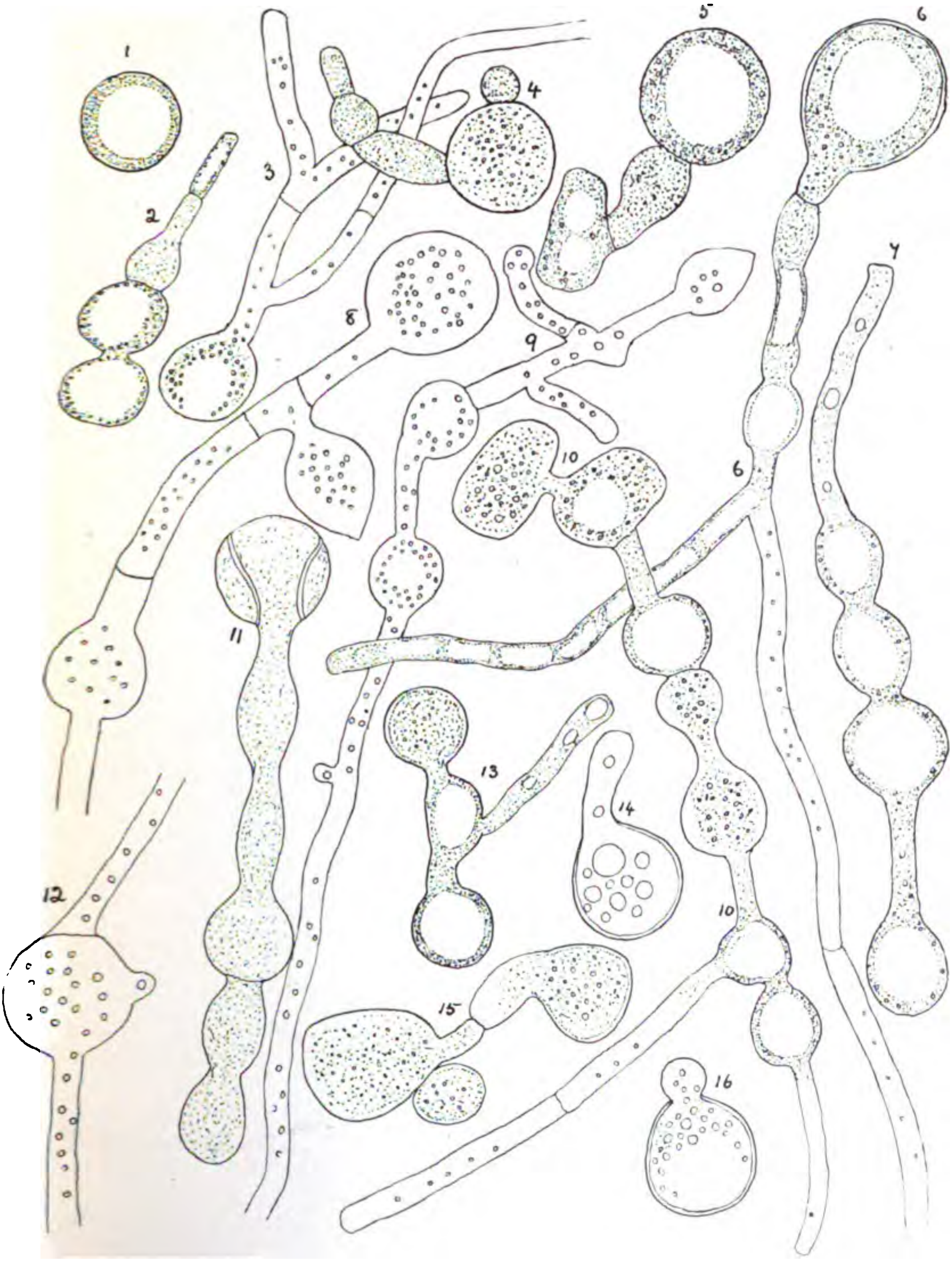
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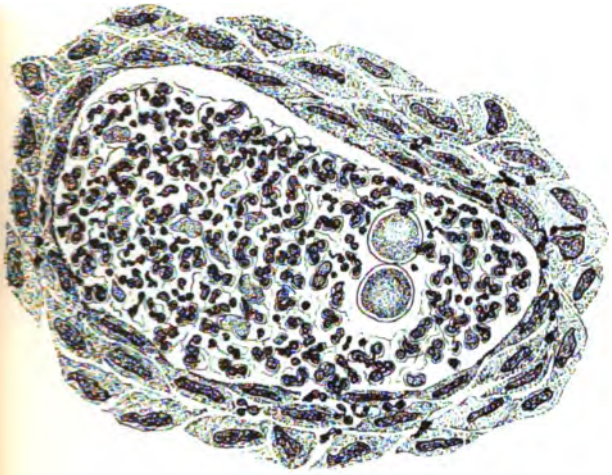

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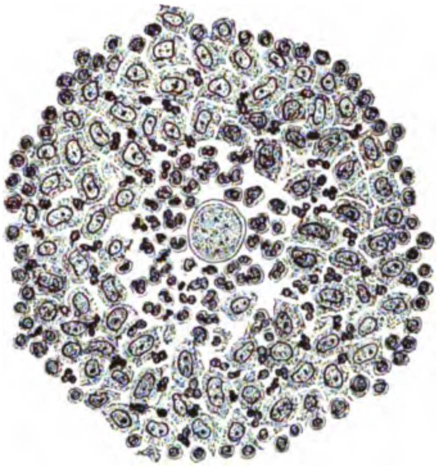

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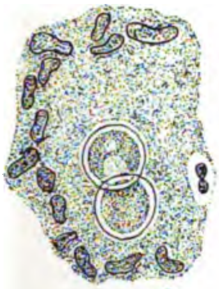

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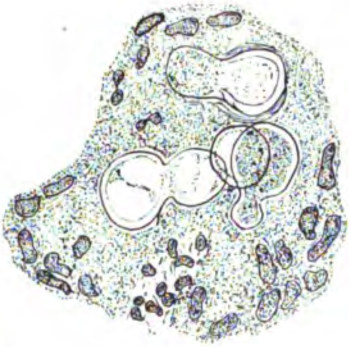

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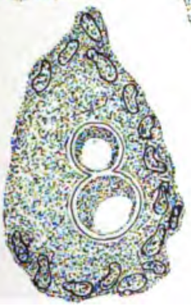

ON THE HÆMOCYTOZOA OF BIRDS.

By EUGENE L. OPIE.

(*From the Clinical Laboratory of the Johns Hopkins University and Hospital.*)

PLATE IX.

Since the discovery of the hæmocytozoa of malarial fever considerable attention has been paid to parasites of a similar nature which have been found in various animals. Those which inhabit the red corpuscles of the blood of birds have excited particular interest because of their close resemblance to the malarial organisms. While numerous European observers, for the most part clinicians and pathologists rather than zoologists, have studied the parasites occurring in the blood of birds, no investigations have up to the present time been made in this country.* Dr. Thayer suggested to Mr. MacCallum and the author to make a study of such organisms as were to be found in the blood of birds captured in the neighborhood of Baltimore. We devoted a portion of the summer of 1896 to this work, which has been done in the Clinical Laboratory of the Johns Hopkins University and Hospital. To Dr. Thayer we are indebted for the many suggestions which he has given us.

History.—Almost ten years before the discovery of the malarial organism by Laveran in 1880, Ray Lankester † had observed in the blood of frogs very small worm-like bodies free in the plasma. Butschli,‡ in 1876, mentions the fact that he has frequently found in the red corpuscles of frogs, peculiar bodies occurring at the side of the nucleus; his figures show that these bodies represent an intracorpuscular stage in the life history of the organism which Lankester saw in the free state. This is apparently the first observation of a parasite within the red corpuscles —a hæmocytozoon.

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* A brief preliminary report of our observations appeared in the proceedings of the Johns Hopkins Hospital Medical Society, *Bull. of the Johns Hopkins Hospital*, March, 1897, No. 72, p. 52.

† *Quarterly Jour. of the Microsc. Sciences*, xi (1871).

‡ *Abhandl. der Senkenberg. naturf. Gesellsch.*, 1876, p. 261.

Ignorant of these observations, Gaule * later described in the red corpuscles of the frog bodies which he speaks of as "Würmchen." These he believed to be formed from the protoplasm of the corpuscle as a result of degenerative changes; he gave them the name *Cytozoa*. Soon afterwards, however, Ray Lankester † established the parasitic nature of these structures and their identity with the organisms which he had previously observed.

Danilewsky ‡ (2) in 1885 described, for the first time, intracorpuscular parasites in the blood of lizards, tortoises and birds. The organisms which he has found in the bird differ markedly from those in cold-blooded animals—frogs, lizards, tortoises. Most conspicuous at first sight is the fact that the latter do not transform the hæmoglobin into pigment granules; other points of difference are, however, not wanting. We have to do at present only with the organisms found in the blood of birds, these most closely resembling the malarial parasites of human beings.

In the numerous articles which Danilewsky has written a variety of forms have been described:

As *pseudo-vacuoles* he describes bodies which appear as clear areas of variable size and shape within the red corpuscle; in the pseudo-vacuoles he finds black granules of pigment, a product of the transformation of the hæmoglobin.

As *pseudo-vermiculi* he describes elongated bodies with an active wriggling motion which are found free in the plasma. He observed two forms: one non-pigmented similar to the organisms found in cold-blooded animals; the other arising from pigmented pseudo-vacuoles which have escaped from red corpuscles.

To the pigmented flagellate organism, which he describes as a distinct species of parasite, he gives the name *Polymitus avium*. These develop as pseudo-vacuoles in the red corpuscles and subsequently undergo the process of flagellation.

At times the flagella become detached and maintain their active motion for a time; to these free flagella he has applied the term *pseudo-spirilla*.

Although Danilewsky had, for a long time, no opportunity of examining malarial blood, he was much impressed with the similarity between the organisms which he had observed and those which had been described by Laveran, and Marchiafava and Celli. The full-grown parasites, pseudo-vacuoles, in the birds examined by him were approximately half-moon-shaped bodies which suggested the crescents of irregular malarial fever; the flagellate forms differed but little from those found in man. In a short article, published in 1886 (4), he raises the question whether or not these organisms are identical, and answers it in the affirmative.

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* *Arch. f. Anat. u. Phys.*, Phys. Abth., 1880, pp. 57, 375; ibid., 1881, p. 297.

† *Quarterly Jour. of the Microsc. Sciences*, Jan. 1882, p. 53.

‡ References to literature at end of this article.

Grassi and Feletti (12) found, particularly in sparrows (*Passer hispaniolensis*) and in pigeons, what they regarded as two distinct types of organism. One form was the half-moon-shaped parasite already described by Danilewsky; this they believed to be closely related to the crescents of malarial fever. They established the genus *Laverania* to include the two forms to which they gave the specific names, *Laverania danilewskyi* (of the bird), *Laverania malariae* (of man).

The second type of parasite, described for the first time by them, was a smaller organism of irregular shape occupying one end of the corpuscle. This body they regarded as closely related to the amœboid forms of the malarial parasites; they established, therefore, the genus *Haemamoeba* to include them. They find in birds two common species of these small, irregular parasites, which they have termed the *Haemamoeba subpraecox* and *Haemamoeba relicta.* They have further described a third type of organism which they observed in a single bird; to this they gave the name *Haemamoeba immaculata,* owing to its freedom from pigment granules.

Celli and Sanfelice (1) have also attempted to bring the parasites found in the blood of birds into conformity with those of the malarial fevers. They base their classification of the parasites which they have observed upon the rapidity with which these organisms undergo their cycle of development. They find:

(1) Parasites with slow development, reproducing after eight days of growth. Morphologically these are the half-moon-shaped bodies of Danilewsky. Celli and Sanfelice think that they correspond to the quartan organism of man.

(2) Parasites with a shorter period of development.

(3) Parasites with rapid development.

Of the two latter varieties they could not determine the exact length of the cycle of development. Morphologically they are irregular bodies lying at one end of the corpuscle. They differ from one another, according to Celli and Sanfelice, in the size of the sporulating form, which in those with quick development is smaller and divides into fewer segments.

The second variety of parasite, that with a cycle of development of mean duration, they think, corresponds to the tertian organism; the third variety, that with rapid development, to the organism of æstivo-autumnal fever. Although Celli and Sanfelice insist upon an analogy between these forms and those of man, they do not assert that they are identical.

They found one or more of these varieties of organism in various species of birds; two varieties may co-exist within the same host. In some instances, where they found for a number of days but one variety, there appeared later a second, which became eventually far more abundant and finally replaced the first. They are in fact inclined to believe that one form can change into another; that they are dealing with a single polymorphous parasite. They have never succeeded in transferring infection from one bird to another of a different species. Hence they come to the conclusion that despite the great morphological similarity between the parasites of different birds they are yet separate organisms, each species of bird having its own variety of parasite which will flourish in this species alone.

Danilewsky (7) has attempted to demonstrate the existence in infected birds of symptoms analogous to those of the malarial fevers. He describes an acute and a chronic "malarial" infection in birds. The acute condition is characterized by rise of temperature, loss of weight and general symptoms of illness. In the chronic infection there is no rise of temperature; after death, however, the spleen is found to be much enlarged and pigmented. The acute infection is caused, he thinks, by the small irregularly-shaped parasite described by Grassi and Feletti; the chronic by the half-moon-shaped bodies. In his later articles Danilewsky does not speak with definiteness of the identity of the birds' parasites with the malarial organisms of human beings; he maintains, however, that the varieties described are very similar if not identical.

Labbé (20), more recently, has carefully studied the intracorpuscular parasites of the blood of vertebrates from a purely zoological point of view. He classifies the various organisms that have been described by himself and previous observers according to structural peculiarities. In birds' blood he recognizes the two types distinguished by Grassi and Feletti. The names which he has assigned to them do not imply any necessary correspondence with the malarial parasites of man. To the small irregular forms he gives the generic name, *Proteosoma*. Of this genus he finds but a single species, *Proteosoma grasii.* To the elongated forms, which lie curved about one side of the nucleus of the corpuscle, he gives the name *Halteridium.* Of these also he finds but a single well-marked species, *Halteridium danilewskyi.*

A large number of inoculation experiments have been performed. Celli and Sanfelice, Laveran and Labbé claim to have successfully inoculated organisms from the blood of one bird into another of the same species. Grassi and Feletti were never successful in such attempts. Di Mattei (11) has more recently performed a large number of inoculation experiments with pigeons. He injected by various methods blood of infected birds into those not infected. In 83 experiments he obtained universally negative results. He injected blood of malarial patients into 16 pigeons with no consequent infection. The same result was obtained in four instances in which he injected the blood of infected birds into the arm veins of healthy men. He concludes, therefore, that, though the parasites of birds morphologically resemble the organisms of malaria, they are essentially different.

PERSONAL EXPERIENCES.

Our studies were carried on in the months of June, July, August and September, 1896. One hundred and twenty-five birds were examined. Blood was drawn from the wing, and both fresh and dried and stained specimens were studied.

About one hundred birds were examined which had been captured in the neighborhood of Baltimore. Of these the greater number, eighty, were English sparrows (*Passer domesticus*); of the remainder, twelve were red-wing blackbirds (*Agelaius phoeniceus*). The others belonged to a variety of species. The great majority of these birds were obtained from places outside the city and near the Patapsco River or Chesapeake Bay, districts notoriously malarious. Of the eighty English sparrows twenty-one were caught in the city on the Hospital lawn; none of these showed infection. Of the remaining fifty-nine sparrows one in twelve was infected. These figures are almost too small to offer even a suggestion as to the influence of locality on the frequency of infection.

Among the red-wing blackbirds infection seems much more common; of twelve birds, six were infected.

In fifteen birds altogether the blood showed intracorpuscular parasites in varying abundance. Among these birds the following species were represented: *Passer domesticus, Agelaius phoeniceus, Melospiza georgiana, Melospiza fasciata.* It is evident that the observations were not made upon a sufficiently large scale to arrive at any very general conclusions as to the species prone to infection, or as to the local conditions favoring infection. Nevertheless, they indicate, probably with sufficient accuracy, the commonest varieties of parasites found in this locality.

While at his home in Dunnville, Ontario, not far from Toronto, Mr. MacCallum examined a number of birds. Of twenty-five, in five were found intracorpuscular parasites. At present there is little if any malarial fever in this locality, although at one time the disease was more common. Of two great-horned owls (*Bubo virginianus*) examined one was found to have a most severe infection. In four of five crows (*Corvus americanus*) the blood contained similar organisms. The birds whose blood was negative included a few individuals of a variety of species.

Types of Organism. Two varieties of organism may be distinguished among the intracorpuscular parasites which we have studied. These correspond to the two forms described by Grassi and Feletti and subsequent observers. Characteristic of each group is the morphology of the full-grown organism. In the one an irregularly shaped body, containing pigment granules, occupies one end of the red corpuscle, while the nucleus of the corpuscle is displaced from its normal central position into the opposite end. The other group is characterized by the fact that the full-grown parasite is an elongated pigmented body lying along one side of the normally situated nucleus and curving more or less over its two extremities; this is the so-called halter-shaped form—*Halteridium* of Labbé. Both develop from very small, non-pigmented, approximately spherical bodies.

In the first variety as the body grows it increases in size gradually in all dimensions, and in growing the parasite exerts sufficient force to cause the displacement of the nucleus. In the second, whether as a result wholly due to peculiarities of the organism or to forces acting within the corpuscle, such, for example, as resistance to the displacing of the nucleus, growth takes place more rapidly along that axis which corresponds with the long diameter of the corpuscle. We may distinguish, then, according to their method of growth, two forms, one irregular and the other elongate. This is their most conspicuous difference; other peculiarities of the two forms are, however, not lacking.

In the majority of infected birds either one or other form was observed alone. Nevertheless, in three birds the two varieties were present at the same time. Hence the possibility suggests itself that the two forms may represent different phases in the life history of the one organism. This view, as we have mentioned, has been supported by Celli and Sanfelice. In birds where one or the other form is present it is, however, possible to trace the whole cycle of development from hyaline non-pigmented bodies to full-grown organisms; it cannot be doubted that the full-grown parasites of both forms segment to form new hyaline bodies. The cycle of development is very different in the two forms and leaves little ground for the belief that one form may appear as a stage in the development of the other. Moreover, in the human being where the multiplicity of species of the intracorpuscular parasites, though not absolutely undisputed, still rests upon much stronger evidence than does that of the organisms of birds, the possibility of an infection with two kinds of malarial parasites at the same time is well known. The frequency of such infection in this locality, for example, is shown by the fact that out of 542 cases of malarial fever studied by Thayer and Hewetson,* there were eleven instances of double infection. The three instances above mentioned, in which the two forms were simultaneously present in the same blood, occurred in blackbirds. Of the blackbirds which we examined one-half contained intracorpuscular parasites. It is not surprising, then, that, where infection is so common, combined infections should be more frequent. Among the English sparrows, of which only one in fifteen was infected, double infection was not observed. For these reasons, then, we believe the two forms to be distinct, and shall therefore consider them separately.

Irregular Parasite (*Proteosoma of Labbé*). The earliest phase of development of this organism is represented by an approximately round, clear body situated within the red corpuscle. Since the body is refractive and gives no indication of amœboid movement, it is hardly to be distinguished in the fresh blood from the vacuoles, which are not infrequently found in the red corpuscles. Nevertheless, in specimens of blood, dried, hardened and stained with eosin and methylene blue according to the method of Romanowsky, modified somewhat by Heppener,* the earliest stage of the parasite is clearly recognizable as a very small body of an oval, round or slightly irregular shape (Plate IX, Fig. 1). The way in which these structures stain is fairly constant; the peripheral portion takes a rather deep blue color, while within this blue rim the substance of the parasite is almost completely unstained.

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* *The Johns Hopkins Hospital Reports*, Vol. V.

As the parasite grows larger it acquires pigment, at first a fine black granule situated within the external staining portion of the organism (Plate IX, Figs. 2-5). As the parasite grows, other granules appear. These are, in the great majority of cases, collected into a more or less loose clump, situated as a rule near the periphery of the body; at times, indeed, one sees a particle of pigment lying apparently upon the surface of the parasite. In the neighborhood of the main clump there are usually one or more isolated granules. In the large forms these may sometimes upon careful observation be seen to have a slow motion away from or towards the main mass; they may merge into it, while the clump itself may slowly change its form, presumably by a rearrangement of the constitutent particles. This collection of the pigment into a single clump in the half- and full-grown forms is almost characteristic of the irregularly shaped organisms; at times, however, one sees parasites with discretely scattered pigment granules. The shape of these pigmented bodies is variable; short blunt processes are very frequent. Although we have carefully watched these organisms in fresh blood for a long time, both at room temperature and on the warm stage at 40-41° C., we have not been able to convince ourselves of any change in their shape. Nevertheless, the irregularity in the shape of the bodies and the frequency with which processes are seen, make it probable that a very slow amœboid movement does occur.

In stained specimens the distinction between the peripheral deeply stained rim and the central unstained portion is lost in these larger forms; the whole body takes a fairly deep color. The stain is, however, not uniform, the body having a somewhat granular appearance; we have not seen the definite granules of staining material which can be made out in certain varieties of the elongate organism (p. 92). Often one may distinguish an approximately round, sharply marked off area, which is almost unstained at times. This may contain one or more faintly staining bodies. This structure, possibly always present in the larger forms, though obscured in some instances by a too deep stain, is what has been described as a vesicular nucleus containing nucleoli.

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* *Med. Pribav. K. Morsk. Sborn.*, Jan. and Feb., i (1895), 67.

The growing organism, which at first may be situated in any part of the corpuscle, comes to occupy one or the other extremity. Not infrequently two parasites, or it may be more, are present in the same corpuscle. In such cases they are almost always of the same size; occasionally, however, one is much larger than the other. As the parasite increases in size a change takes place in the position of the nucleus of the corpuscle; it is deflected from its normal longitudinal and central position until it not infrequently comes to lie almost or quite at right angles to the long axis of the corpuscle and at the same time nearer one extremity.

One would suppose that the displacement of the nucleus was the mechanical effect of the growth of the parasite, and, in fact, in many cases the parasite fills the whole of one extremity of the corpuscle with one of its sides flattened out against the nucleus which lies crowded transversely into the other extremity. Not infrequently, however, as Labbé has pointed out, a small parasite may be at one end of the corpuscle, while the nucleus is displaced into a transverse position, although it is touched at no point by the parasite (Plate IX, Fig. 2). The explanation of the process in such cases does not seem, then, to lie altogether in a mechanical displacement of the nucleus, but apparently involves a consideration of the internal structure of the red corpuscle and of the forces that hold the nucleus in place. Upon these points, beyond the obvious fact that there is a marked disturbance of such forces, we can say nothing.

It is interesting in this connection, that one not infrequently may come across parasites surrounded by a rim of red blood corpuscle from which the nucleus is absent, having been apparently extruded (Plate IX, Figs. 6 and 7); this picture we have observed only with the variety of organism now under consideration. In such cases the remains of the corpuscle are somewhat distorted; often one extremity is drawn out into a point. The stroma of the corpuscles containing the half- and full-grown forms is not decolorized, nor is any alteration of the nucleus to be seen.

With the irregular organism the whole process of segmentation, which is closely similar to that of the malarial parasites of man, takes place in the circulating blood. Bodies representing the first stages of the process are at times met with in great abundance. The parasite assumes an approximately round form; the pigment collects into a solid block which occupies the centre of the body. The outline of the parasite is not perfectly even, but shows more markedly, in proportion as the process has advanced, indentations at regular intervals, which give the whole body somewhat the appearance of a rosette. Situated near the periphery is a circle of minute refractive dots, each of which occupies a position midway between two of the peripheral indentations. The indentations at the periphery become more marked and the organism divides into a variable number, roughly speaking five to twenty small bodies, which assume a circular outline. The size and shape of the segmenting bodies vary. In the larger forms within the peripheral row an internal row of segments about the pigment clump may be found.

Very frequently the corpuscle containing these large bodies shows certain changes; there is some decolorization of the stroma; the corpuscle becomes less oval, the short diameter being increased at the expense of the long; the nucleus of the corpuscle also is often apparently swollen, becoming round and less elongated. Finally, the newly formed bodies become free, and in fresh specimens of blood solid pigment clumps surrounded by groups of small spherical bodies may be seen free in the plasma. Stained by the method of Romanowsky, the segmenting bodies always take a deep color. When the process has advanced almost to division into separate segments, the body may be seen to consist of a number of approximately round, clear areas surrounded by deeply stained protoplasm; within the clear spaces may be seen blue specks which represent possibly the refractive dots of the fresh body (Plate IX, Fig. 9). After complete segmentation there result a number of round bodies staining at the periphery and clear in the centre, identical in appearance with the youngest intracorpuscular parasites (Plate IX, Fig. 9).

As a rule the segmenting bodies correspond in size with the larger full-grown organisms, filling somewhat more than one-half of the corpuscle. Not infrequently, however, one may see in the same blood segmenting forms hardly half this size. This suggested the possibility that we may be dealing with two distinct organisms. Celli and Sanfelice think that the larger and smaller segmenting bodies belong to separate varieties; in fact, this is, as has been mentioned, the most important point of distinction between their second and third varieties of parasites, those with cycles of development of respectively mean and rapid duration. Labbé, on the other hand, because he finds the larger and smaller forms always associated, while, at the same time, others of intermediate size occur, comes to the conclusion that they are forms of one organism, a parasite which under certain circumstances may sporulate at a period somewhat earlier than usual in its life history. This explanation is in accord with what we have observed.

From analogy with what we know of the tertian and quartan parasites of human malaria it is natural that one should suspect that these organisms might, in a similar manner, undergo a cyclic development in groups, all the members of each group being in approximately the same phase of development. This, however, is apparently not the case. In a specimen of blood in which the parasites are fairly abundant one finds as a rule nearly all the forms that have been described, though not infrequently one form predominates. For this reason we have been able to arrive at no definite conclusion as to the length of the cycle of development.

In addition to the full-grown organisms, which correspond in size to the largest segmenting bodies, certain other forms of a characteristic appearance are usually to be found. These are as a rule much larger than any of the segmenting bodies and are apparently not destined to segment, at least in the ordinary way. The nucleus of the containing corpuscle is pushed to the extreme periphery; the size of the parasite is usually such that the corpuscle is distorted. Often one finds the parasite surrounded by a rim of corpuscle from which the nucleus has been extruded. As a rule the corpuscle or what is left of it is decolorized. These bodies are much less refractive than the smaller forms and stand out, therefore, much less distinctly from the corpuscle. The pigment is not collected into a clump, but is distributed throughout the organism as discrete granules. In specimens that have been fixed and stained with eosin and methylene blue, best by the method of Romanowsky, these bodies remain almost completely unstained (Plate IX, Fig. 7); occasionally a stained peripheral rim may be made out surrounding a large unstained central area.

These are, apparently, the bodies that Grassi and Feletti have described as a separate species of organism under the name *Haemamoeba relicta*. They believed them to segment as do the other forms. Celli and Sanfelice, on the other hand, have found them only in connection with their parasites with rapid development and regard them as forms analogous to the crescents of æstivo-autumnal fever.

We have found these bodies associated with the irregular type of organism whenever the parasites were at all abundant. The fact that they are as a rule larger than any of the segmenting bodies, as well as the fact that they stain very faintly, while the segmenting bodies always contain much chromatophilic substance, is in favor of the view that they are sterile forms.

In specimens of blood containing only the irregular form of parasite we have a number of times met with flagellate bodies very closely resembling those seen in human malaria. A round extracorpuscular body, usually lying beside the free nucleus of a red blood corpuscle, is seen to acquire an active vibratory motion. This motion is due to a number of actively motile flagella which have appeared at the periphery. The flagella are similar in appearance to those which occur in the malarial parasites of human beings. They are several times the length of the diameter of the body from which they arise. At their extremities they often show slight olive-shaped swellings. Alterations in the shape of the body of the organism are common; a constriction may apparently divide it into two circular bodies touching at but one point (Plate IX, Fig. 10), while later it again may take on its original circular shape. In time the flagella disappear and the body becomes motionless. The presence of the free nucleus beside the extracellular body indicates, in view of what is often seen to occur with the elongate parasite (p. 94), that the organism may have escaped from the corpuscle after the preparation of the specimen. The size of these extracellular parasites which develop flagella, and the fact that they are not so refractive as the smaller intracellular bodies, suggest that they are possibly identical with the large palely staining bodies with discretely distributed pigment granules.

The above described parasites we have found in the English sparrow (*Passer domesticus*), swamp sparrow (*Melospiza georgiana*) and red-winged blackbird (*Agelaius phoeniceus*). No differences were made out between the organisms found in these different species of birds.

Elongate Parasite (Halteridium of Labbé). The second variety of parasite which we have observed, the elongate organism which lies curved alongside the nucleus, is markedly different from the irregular form which we have just considered. Since we have found that distinct morphological differences were to be made out between the parasites of this variety present in different birds, as a type that variety will be described which was found in the greatest number of hosts. In the English sparrow (*Passer domesticus*), song sparrow (*Melospiza fasciata*) and crow (*Corvus americanus*) were found organisms morphologically indistinguishable. These may be regarded as typical examples of the halter-shaped parasite.

The youngest non-segmented organisms closely resemble corresponding forms of the irregular parasite; pigment soon appears as small brownish granules. The small forms vary in shape, usually they are round or oval. We have not observed amœboid movement, though very short processes are often present. These young forms stain only in their peripheral portions, as do the similar stages of the irregular parasite; the central part, which contains no pigment, remains unstained (Plate IX, Fig. 11). As the parasite grows it becomes elongated. Having reached a certain size it appears to adhere closely to the nucleus of the corpuscle. When somewhat longer than the nucleus the parasite is of semilunar shape with pointed ends hugging the nucleus (Plate IX, Fig. 12). With further growth the extremities of the parasite become more bulky and rounded and finally fill almost the whole of both ends of the corpuscle. The full-grown body then lies along one side of the normally placed nucleus and curves over its two extremities (Plate IX, Figs. 13-14). The pigment of these large forms appears as very coarse oval highly refractive granules of a yellowish-brown color, distributed irregularly throughout the parasite or collected towards the two ends. The corpuscle containing the very large forms is usually somewhat decolorized, but otherwise looks normal.

Additional information as to the morphology of the organism can be obtained from specimens fixed and stained. The method which we have used most extensively is that of Romanowsky. The material which in the young forms takes up the stain increases in amount with growth, so that in the large bodies it is scattered throughout the parasite. These large parasites take a deep stain which is not, however, homogeneous, but results from the presence of a number of small oblong chromatophilic particles; these chromatophilic granules are well seen in specimens fixed by heat, stained with safranin and partially decolorized in alcohol.

In forms but little longer than the nucleus of the corpuscle there becomes visible an approximately circular, clear-cut area, which stands out conspicuously from the fact that it remains unstained. Within this pale area one or more small bodies may at times be seen which, by the methods employed, take a very faint stain. Various observers have described such a structure as a vesicular nucleus containing nucleoli.

Besides these deeply staining forms, and at times in almost equal abundance, we have found full-grown bodies which remain almost completely unstained (Plate IX, Figs. 14-16). The longest of these are somewhat larger than the deeply staining bodies. The pigment granules are situated in the two extremities; this peculiarity is explained by a further fact which staining brings out. The whole of the middle portion of the organism is occupied by a very large, clear, almost completely unstained space, sharply marked off from an exterior layer, thickest at the two ends, which takes a faint blue stain. The sharp outline and position of the central clear space indicate that it corresponds to the so-called vesicular nucleus of the other forms. The outer faintly staining layer represents apparently the body of the parasite containing a very small amount of chromatophilic substance. It is in the latter portion that the pigment is situated, and since this portion is thickest at the extremities of the parasite, it is here that the pigment tends to collect.

When the blood is drawn large numbers of these swollen, palely staining forms quickly assume a circular outline and escape from the containing red corpuscle. In stained specimens of these circular bodies (Plate IX, Fig. 16) the central unstained area is surrounded by the external palely staining material which now forms a rim of uniform thickness. In this peripheral rim lies the pigment. In many of these bodies, which have become completely extracellular, the distinction of internal non-staining area and external palely staining material is lost and the pigment is scattered throughout the body.

The question arises whether these non-staining bodies are forms which after maturing have, by a sort of degenerative process, lost their affinity for stains, or are forms which have never acquired any considerable amount of chromatophilic substance. In favor of the latter hypothesis is the fact that smaller forms which take but little color are frequently found in the same specimen with the larger bodies.

Reproduction of the elongate form of intracorpuscular parasite does not, according to our observations, take place in the circulating blood. Neither in the English sparrow, song sparrow nor crow infected with this parasite have we found bodies showing any indication of segmentation. The bone-marrow and internal organs of these birds were not, however, examined in the fresh state.

Labbé is the only observer, so far as we know, who has definitely described the segmenting stage of the halter-shaped organism. In the lark, particularly in the bone-marrow and spleen, he found segmenting bodies. According to him the nucleus of the parasite divides into two, the daughter nuclei occupying the two extremities of the organism. The two extremities containing the secondary nuclei then divide, each into from six to fifteen segments. We have not observed this process, although in one or two instances in the owl (*Bubo virginianus*) we have seen parasites with two of the so-called nuclei.

In the bone-marrow of a swamp sparrow (*Melospiza georgiana*) infected with elongate organisms, which to be sure differ somewhat from those which we have just described in the English sparrow, song sparrow and crows, we have, however, encountered bodies in process of segmentation. The organisms, as seen in preparations of bone-marrow teased in normal salt solution, had assumed a round form with the pigment collected into a central block, and had divided into a number of round segments arranged about the pigment mass.

From analogy with the segmenting bodies of the irregular parasite which stain well, one would suppose that it is the deeply staining bodies of the elongate parasite that segment, while for the same reason it seems improbable that the palely staining forms which are characterized by swollen vesicular body undergo segmentation.

The process of *flagellation* presented by the elongate organism is a most remarkable phenomenon. In birds in which these organisms are abundant the whole process may be followed with the greatest facility; in almost every field of the microscope (Leitz, Obj. oil im. 1/12; Oc. 3) one or more flagellate bodies may be observed. To see the whole process the specimen of blood must be prepared and brought under the microscope with considerable rapidity. Certain of the full-grown bodies which lie curved along one side of the nucleus of the corpuscle are soon seen to collect themselves into an oval, then into a circular form, causing a marked bulging protrusion from that side of the containing corpuscle. As one watches the corpuscle its body disintegrates; the hæmoglobin seems to fade away under the eye and a round organism is left lying beside the nucleus of what was shortly before the containing corpuscle. Very soon the round extracellular body acquires an active vibratory motion as the result of the presence of two, three, four, or even more, very active flagella, which suddenly make their appearance at the periphery (Plate IX, Fig. 17). The pigment granules take on a very lively dancing motion. Changes in the shape of the body of the organism often occur. As with the flagellate forms of the irregular variety, a constriction may divide the parasite into two circular bodies connected at but one point. After a while individual flagella not infrequently become detached and float away, continuing their active serpentine movements. After persisting for some time the flagella become less active and finally disappear, by exactly what process cannot be said in every case. The pigment loses its motion.

The liberation of the intracorpuscular parasites appears to be the direct consequence of the changes which take place in the blood as a result of abnormal conditions produced in making the specimens, such as, for example, the rapid reduction of the temperature of the blood, the chemical changes in the plasma accompanying coagulation and the changes resulting from exposure of the blood to the air. It cannot be definitely determined whether the liberation of the parasite is primarily the result of disintegration of the containing corpuscle whose resistance to external changes has been weakened by its presence, or whether it may be due to the action of the parasite which, meeting with the new conditions, contracts into a globular form, and in so doing breaks from the corpuscle. It may be said that in specimens of birds' blood, however carefully made, a certain number of corpuscles which do not contain parasites disintegrate; the hæmoglobin is dissolved in the plasma and the nucleus alone remains. Where little care is used in the preparation of the specimen large numbers of these free nuclei are seen. Where organisms are contained in one of these disintegrating corpuscles they apparently become free, assuming a round form even though they have not reached the size of those which ordinarily escape. In such a case certainly the liberation of the organism is not primarily the result of its own activity, but of the distintegration of the corpuscle. A similar explanation probably holds for the larger forms.

A certain number of the organisms that become free, but by no means all, develop flagella. We have, it will be remembered, described two forms of full-grown organisms: the deeply staining bodies with small nucleus-like areas and the palely staining forms with the large vesicular structure, which are constantly present in the blood of birds infected with the elongate type. Do both of these forms develop flagella? Since this question has suggested itself to us we have not been able to obtain a bird with sufficient number of forms capable of developing flagella to enable us to arrive at any conclusion.

If blood be allowed to stand some time before the dried preparation is made, it will be found that both the pale and the deeply staining parasites become free. This is, however, of no especial significance; under such conditions many non-parasitiferous red corpuscles also disintegrate. As far as we have been able to judge, the palely staining forms are the first to become free. Sakharoff (26), who with Danilewsky thinks that the flagellate forms are a separate species of organism, states that the organisms which flagellate are distinguished from those that do not by the possession of a very large nucleus. These bodies with large nuclei would appear to be identical with our swollen palely staining forms, which we have been rather inclined to regard as the variety which develop flagella.

Varieties of the Elongate Parasite. There is considerable diversity of opinion among observers as to the existence of distinct species of the organisms under consideration. Celli and Sanfelice observed certain special differences in the size and shape of the elongated parasites. For example, in some birds the extremities of the full-grown forms were more blunt than in others; in the barn owl (*Strix flammea*), and in the brown owl (*Syrnium aluco*), the organisms contained numerous conspicuous refractive particles scattered between the pigment granule.

Labbé, in his *Halteridia* of the lark, chaffinch, starling and jay, found differences in shape and refraction in different birds. These authors do not, however, emphasize morphological differences as indicating the existence of separate species of organisms. Celli and Sanfelice, however, think their failure to transfer organisms by inoculation from one species of bird to another, indicates that there are distinct species of parasites peculiar to the different hosts.

Labbé finds but one species of Halteridium which, in view of unsuccessful inoculation from one species of bird to another, he thinks is composed of numerous varieties peculiar to the special hosts. Little reliance can, however, be placed upon unsuccessful inoculations from one species of bird to another as pointing to specific differences, since more recently Di Mattei, as already mentioned, has in a large number of carefully conducted experiments wholly failed to transfer infection from bird to bird of the same species.

The elongate parasites which we have encountered have presented in different birds distinct structural peculiarities which in certain cases are very striking. In several instances, however, we have observed similar differences in the organisms of a single bird. Hence, even though the morphological differences between the parasites are greater than those existing between the tertian and quartan organisms of human malaria, the lack of extended observations makes it impossible to establish distinct species. Nevertheless, we will call attention to the chief varieties which we have noted.

(1) In an English sparrow and a song sparrow obtained near Baltimore, and in three crows from near Toronto, Canada, were found organisms in every respect identical (Plate IX, Figs. 11-17). These, selected as an example of the halter-shaped organism, have already been described. They present certain distinctive features. When the young forms have reached a length but little greater than that of the nucleus of the corpuscle, unlike the other varieties, they adhere closely to the nucleus. The extremities of the organism, at first pointed, grow over those of the nucleus, hugging them closely. At this time the body is crescentic; later its two ends become rounded and bulky. The most striking feature, however, of this variety is the pigment. This is scattered through the organism as very coarse, yellowish brown, highly refractive oval granules; these in the full-grown forms are several times the size of those usually found. The large palely staining forms with swollen vesicular structure are found in great abundance. Flagellate bodies also are seen here in great numbers.

(2) In a great horned owl (*Bubo virginianus*) an organism (Plate IX, Figs. 18-22) was observed which differed markedly from that above described. In common with the other varieties it begins its growth as a small non-pigmented body, situated as a rule in one or

other end of the red corpuscle. With increase in size it often comes to occupy a large part of the extremity and may be so large as to slightly displace the nucleus, never, however, pushing it into the opposite end. At the same time growth proceeds longitudinally along one side of the nucleus toward the other end of the corpuscle. When full-grown the organism occupies every available portion of the corpuscle, displacing the nucleus laterally. The pigment consists of numerous small spherical granules which are almost black.

In fresh specimens in the half-grown and full-grown forms there may be seen between the pigment granules numerous, highly refractive, spherical particles. In specimens stained with methylene-blue and eosin these bodies appear as completely unstained round areas within the blue ground of the organism. The vesicular body described as a nucleus stands out clearly in stained specimens; within, one or more palely staining particles may be made out. Palely staining parasites are abundant. Flagellate forms with from three to five flagella are numerous. Irregularity in the shape of the flagellate body is common; bud-like processes often appear at the periphery and may give origin to flagella.

(3) In a swamp sparrow (*Melospiza georgiana*) was found an organism (Plate IX, Figs. 23 and 24) markedly unlike those already described. When full-grown it appears as a rather slender body of almost uniform width lying along the periphery of the corpuscle, at one side of the nucleus but not touching it. The border of the parasite toward the nucleus has an irregular appearance. Pigment is present in fine round granules. Conspicuous refractive bodies are not present. Although intracorpuscular organisms were seen to assume a circular shape and become free, flagellation was not observed, possibly on account of the small number of parasites present. In the bone-marrow of this bird, as already mentioned, segmenting bodies were found.

(4) In several red-wing blackbirds (*Agelaius phoeniceus*) there were seen organisms (Plate IX, Figs. 25 and 26) resembling somewhat those of the last mentioned form. They differ from these, however, in not being so slender and in their lack of uniformity in width. As

a rule they do not lie along the periphery of the corpuscle, but are often close to the nucleus. Those forms which have not completed their growth show as a rule a somewhat club-like shape, one end being more bulky than the other; growth apparently takes place along the side of the nucleus from the larger end as a starting point. In the largest forms both ends are clubbed. The pigment is in small spherical granules not infrequently collected into one or both extremities.

(5) In one red-wing blackbird (*Agelaius phoeniceus*) occurred an organism (Plate IX, Figs. 27-29) which differed from that last mentioned. The extremities of the larger forms are not clubbed, but are pointed, curling about the ends of the nucleus of the corpuscle, and with further growth approach one another on the side of the nucleus opposite to the main bulk of the parasite. In certain cases the two extremities meet one another and fuse, thus forming a complete ring surrounding the nucleus. Pigment is present as round granules, usually scattered throughout the organism, at times collected in a more or less definite clump. Associated with this variety of organism is a form (Plate IX, Fig. 29) very similar to bodies which Kruse (15) has described and pictured, regarding them as, in all probability, segmenting forms. They are seen in specimens stained by the method of Romanowsky as elongated bodies lying along one side of the nuclei; the pigment is collected into a fairly compact mass which occupies the middle portion. The body of the parasite is composed of a number of round areas which take no stain, surrounded by stained protoplasm. The whole has the appearance of being composed of a number of bodies with central, non-staining area and a peripheral staining rim. Whether or not these are really segmenting bodies cannot be definitely stated.

The above mentioned differences among the organisms observed certainly suggest the possibility of the existence of distinct species. On the other hand, it may be that a single organism is modified by peculiarities in the conditions which it meets in different species of host or in the same host under varying conditions. But even if the above described peculiarities do not indicate specific differences among the organisms concerned, they are nevertheless worthy of note as evidence of a very remarkable polymorphism.

DESCRIPTION OF PLATE IX.

The figures, those of flagellate forms excepted, are drawn from specimens stained with methylene blue and eosin by the method of Romanowsky (modified by Heppener). The figures of flagellate forms are drawn from fresh specimens of blood. Leitz, Oc. 3; Obj., oil im. $\frac{1}{12}$.

Figs. 1-10.—Irregular parasites found in the English sparrow, swamp sparrow and red-winged blackbird.

Figs. 11-17.—Elongate parasites, variety (1), found in the English sparrow, song sparrow and crow.

Figs. 18-22.—Elongate parasites, variety (2), found in the great horned owl.

Figs. 23 and 24.—Elongate parasites, variety (3), found in the swamp sparrow.

Figs. 25 and 26.—Elongate parasites, variety (4), found in the red-winged blackbird.

Figs. 27-29.—Elongate parasites, variety (5), found in the red-winged blackbird.

For fuller description of these figures see the text.

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PLATE IX.

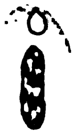
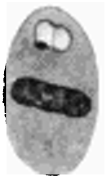


Fig 1



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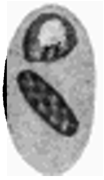

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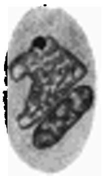

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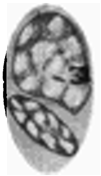

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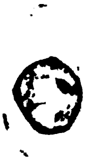

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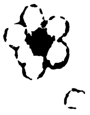

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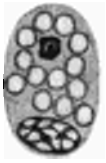

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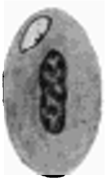

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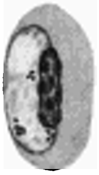

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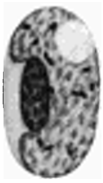

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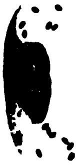

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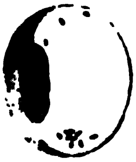

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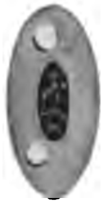

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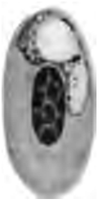

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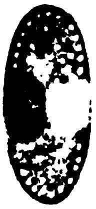

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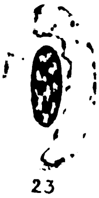

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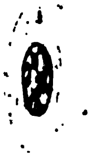

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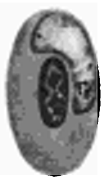

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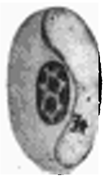

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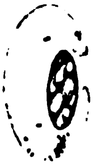

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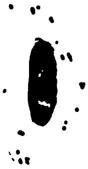

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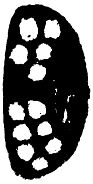

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NOTES ON THE PATHOLOGICAL CHANGES IN THE ORGANS OF BIRDS INFECTED WITH HÆMOCYTOZOA.

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PLATES X AND XI.

Several authors have given brief descriptions of deposits of pigment found by them in the tissues of birds which had for some length of time been infected with hæmocytozoa. Danilewsky, in his "Parasitologie Comparée du Sang," in 1889, speaks of the changes in the hæmopoietic organs and describes macrophages containing pigment masses, parasitiferous and shrunken red corpuscles and the remains of parasites. Again, in 1890, he describes the blackening of the spleen and the enclosure of pigmented cells in large macrophages. He found also similar changes in the bone-marrow.

Kruse (2) in the same year drew attention to the dark color of the spleen, although, owing to his lack of familiarity with the anatomy of birds, he was unable to decide whether or not the organ was enlarged. He found pigment enclosed in the large clear cells in the capillaries of the spleen, liver, lung and bone-marrow; he regarded this as an evidence of the acuteness of the process, since he argues that in a chronic infection the pigment would probably all be contained in fixed cells.

Labbé (1) in 1894 speaks also in a general way of the gross changes in the organs of infected birds.

Our own observations on the pathological changes in the organs of birds infected with hæmocytozoa are based on the results of a study of the tissues of those birds whose blood has been described by Mr. Opie in the preceding paper (p. 79). In a series of about 125 birds autopsies were made in all cases in which infection was found, and for the sake of control in a few which were free from infection. In a number of cases, of which brief accounts are given at the close of this article, the tissues were examined microscopically.

From these descriptions it will be seen that the conditions resulting from infectión with the so-called "Halteridium" and the "Proteosoma" are, roughly speaking, identical. The slight differences will be brought out in the following description of the minute appearances of the various organs.

Liver. The capillaries are not as a rule dilated. In a few instances, however (No. 31), we have observed them in places to be crowded with infected corpuscles which seemed to completely occlude the lumen. Isolated infected corpuscles are seen scattered in the capillaries throughout the liver, being more numerous in the larger vessels. Many of these are well-preserved, but according to the conditions under which the tissues were hardened, the organisms have been, in greater or less proportion, extruded from the corpuscles and lie in the capillaries as pigmented spheres, accompanied by the free nuclei of the red corpuscles. The organisms in the tissues take on a pink stain with hæmatoxylin and eosin, and present a somewhat granular appearance.

The larger masses of pigment seen in the liver tissue are contained in large cells, which vary greatly in size, shape and staining properties (Plate X, Fig. 2). They are very similar to the macrophages described as occurring in the organs from cases of malaria in human beings, and are probably analogous to these. In the liver of other animals essentially identical structures have been found acting as store-houses of pigment; the previous descriptions of their occurrence in birds in hæmocytozoan infections have already been referred to. These cells are round, ovoid or irregular structures, sometimes large enough to completely block the lumen of a capillary. That they are really in the capillaries seems to be definitely proven by some of our sections, and with this view most recent writers agree. Ponfick (12), however, in his article on the " Fate of Pigment in the Animal Body," says that in the liver of animals into whose blood cinnabar has been injected, cells similar to these and containing the cinnabar granules are to be found in the lymphatics of the vessel sheaths or, at any rate, outside the blood-vessels.

He finds also that the endothelial cells of the capillaries take no part in the storing up of pigment, a conclusion quite contrary to that arrived at by us from our observations in birds.

The protoplasm of the macrophage may remain clear and unstained; in other cases it takes on a lilac color in specimens stained in hæmatoxylin and eosin. The nucleus is always vesicular, varying in shape, being sometimes quite distorted, but oftener rounded. In some instances two nuclei have been found. Of all the varied contents of these cells the most striking is the pigment which occurs in at least two distinct forms, as follows: (1) The pigment formed by the organism—blackish-brown granules of fairly uniform size and shape, which occur separately or grouped together in spherical or irregular masses of various sizes, sometimes, apparently, far too large to be contained in a cell. (2) Straw-colored pigment, probably derived directly from the remains of the hæmoglobin which is set free into the blood stream when an infected red corpuscle breaks down and liberates the contained organism. This occurs in irregular particles, sometimes aggregated into masses similar to those just described. Although these sometimes form the only contents of the cell, many of the masses found crowding the macrophages are made up of both varieties of pigment packed together.

In other cases the large cells are filled with small masses of a fairly uniform size, sometimes irregular in outline, sometimes so closely packed together as to take on a polyhedral shape. At first sight they appear yellowish-brown, but on closer inspection are seen to be made up of a faintly colored ground substance with refractive brown granules of pigment—they are in fact shrunken parasites.

Neumann (13) and Bignami (8), in speaking of the varieties of pigment found in the organs in chronic malarial infections, recognized the fact that not only does the malarial pigment give no reaction to the ordinary tests for iron (such as Perl's reaction with ferrocyanide of potassium and hydrochloric acid or the ammonium sulphide reaction), but that, after they have been deposited in the tissues for a certain time, the iron-containing compounds derived from the hæmoglobin also cease to react. This may explain the fact that, in specimens of birds' tissues which were treated with ferrocyanide of potassium, although a hazy blue stain was obtained in the cells and about the pigment, none of the fragments gave a definite blue reaction.

Treated with ammonium sulphide the pigment formed by the organisms is entirely unchanged, while in most cases some of the straw-colored variety is blackened. If, however, after the treatment with the sulphide the section be washed in water and exposed to the air, the blackened pigment becomes decolorized to a certain extent (cf. Quincke (14)), and the malarial pigment is brought out in relief.

Besides these various forms of pigment the macrophages frequently contain infected red corpuscles, which are sometimes unchanged, sometimes shrunken. In others the remains of such corpuscles form a mere debris, consisting generally of the shrunken bodies of the parasites, and finally of the broken-down remains of these with free pigment scattered in the protoplasm. The pigment is often arranged in radiating lines; sometimes it appears to lie in vacuoles. These, however, may be artefacts.

Not only the macrophages but also the endothelial cells of the capillaries may be seen to contain pigment masses (Plate X, Fig. 1). This pigment appears usually in small quantities and occurs at the poles of the nucleus. Occasionally, however, the cell is greatly distended with large pigment masses.

We are still uncertain as to the presence of pigment or parasites in any cells corresponding to Kupffer's cells in man. Many large mononuclear cells, with deeply staining nuclei and protoplasm, which takes on a lilac coloring, are found in the capillaries. Whether these can take up pigment and are to be classed with the macrophages proper we have not determined. On staining with acid fuchsin an occasional white blood corpuscle, of the class which we have for convenience termed rubinophilic cells,* may be found in a greatly swollen condition and containing pigment masses. This is, however, rather the exception, inasmuch as numerous examples of such cells are seen throughout the tissues quite free from pigment or other foreign substance.

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* See page 113.

The liver cells themselves have not been observed to be phagocytic, but a very considerable pigmentation is noticeable in one or two cases (Nos. 103 and 111). Here the liver cells present a ragged vacuolated appearance and contain numerous fine straw-colored granules. On applying the ferrocyanide of potassium and hydrochloric acid or ammonium sulphide stains for iron, a sharp iron reaction is obtained and the liver presents the appearance of a close-meshed network of blue or black lines of pigment granules, according to the method used. In one liver (No. 3) there was extensive fatty degeneration.

As to the effects of the organisms or their products on the tissues but little that is definite can be stated. Necroses have been observed in the organs of several specimens, but their dependence upon the presence of the hæmocytozoa cannot be said to be proven.

We have observed two forms of focal necrosis:

(a) The first, found in the owl (No. 101), in which there was an intense septicæmia, is probably due to the presence of the bacteria. These necroses are described in the account of the autopsy (p. 115).

(b) The necroses of the second variety were found in the liver and, in a modified form, in the spleen of birds with proteosoma infection. They appear to be associated with the presence of certain oval bodies which seem to bear no close relation to the proteosoma itself (Plate X, Figs. 3 and 4). The foci in the liver are small and discrete. In the affected spots there is a complete loss of liver cells as such in the area, the centre of which is occupied by a mass of pink-staining material, which contains fragmented, deeply staining nuclei, with the debris of cells and occasional macrophages enclosing pigment and parasites (Plate XI, Fig. 1).

Among these there are often to be seen cells containing small oval bodies just about the size of the nucleus of a red corpuscle. These bodies take a general lilac color and show in their centres a ring-shaped structure which stains rather deeply with hæmatoxylin. These ovoid structures may occur free in the midst of the debris of cells and nuclei, but are more often contained in cells, where they may be seen in groups of two or more, or in larger collections of from twelve to twenty arranged with the pointed end turned to the centre and calling to mind the appearance presented by the segmenting bodies of a malarial parasite (Plate X, Figs. 3 and 4).

These groups contain no pigment in their interstices and, from their size and general appearance, give rise to the idea that possibly they represent the segmenting stage of a protozoan parasite. This is of course only an hypothesis, and until further material is obtainable the question of their exact nature must remain undecided. Their distribution is not limited exclusively to the neighborhood of the necroses; they may be seen contained in cells elsewhere in the capillaries. They will be referred to later in discussing the spleen.

Spleen. Kruse (2) has described the changes in the spleen in infected birds. He finds that it is of a greyish-black color and contains masses of pigment within colorless cells distributed essentially in the vessels. Judging from the bright color and the great size of the granules, he believes that only one part of this pigment corresponds to that formed by the parasite. The red corpuscles taken up by the leucocytes contribute to the pigment formation, but whether the parasite first kills the corpuscle and the two are then engulfed, or whether the leucocyte swallows both alive, remains a matter of doubt.

Owing to certain peculiarities in the distribution of the pigment in the spleen it is desirable to consider certain details in the normal anatomy of the spleen of birds before entering upon a description of the pathological changes. Wilh. Müller (6) and A. J. Whiting (7) have given the most satisfactory accounts of the structure of the spleen of birds; their observations differ in no essential points from those made by us. The capsule, at least in the smaller birds, possesses no trabecular system; the vessels, however, are surrounded by a certain amount of connective tissue. In the neighborhood of these masses of connective tissue the pigment is especially deposited. At the points of branching of many arterioles spherules of lymphoid tissue are seen placed laterally, sharply marked off from the adjoining tissue by a double row of elongated cells, which Whiting considers to be muscular, but which, since they sometimes take up pigment, seem to us to resemble rather endothelial cells. These do not occur in sparrows.

The further prolongations of the arterioles show here and there changes in the nature of their endothelium, the nuclei of which become large and vesicular. In these places the adventitial layer becomes greatly thickened so as to form an ellipsoidal mass of pink-staining tissue, in which are embedded large vesicular nuclei. Associated with these structures there is a considerable amount of lymphoid tissue poor in capillaries, the round cells of which merge gradually into the large cells of the pulp proper, which runs in bands among the spherules and ellipsoids. These pulp bands are made up of wide capillary spaces which anastomose freely in a network of large pulp cells, characterized by large vesicular nuclei of round, oval or polyhedral form, and with slightly opaque protoplasm which takes a pink stain. These cells are most closely aggregated at the margins of the bands of pulp, where in hæmocytozoan infections they become conspicuous from their actively phagocytic properties. Sometimes, as in case No. 101, they become so loaded with pigment that the pulp bands appear as a black network on section of the spleen (Plate XI, Fig. 2). They exist in numbers directly under the capsule, where again they may contain a conspicuous collection of pigment. The extent of the pigmentation varies greatly, as will be seen from the autopsy reports. The nature of the deposit, however, and its distribution is similar in all, the pigment resembling exactly that described as occurring in the liver, and giving the same staining reactions.

The proportion of the varieties of pigment differs to a certain extent in the different specimens; for example, in No. 104 the pigment is mostly yellow, while in Nos. 5 and 103 it is mainly of the dark variety; this is, however, possibly dependent on the duration of the process, the yellow pigment being, as Bignami has suggested, changed by a local reaction into a dark material which does not respond to ordinary tests for iron. Almost all of this pigment is contained in the large cells mentioned above as forming the groundwork of the vascular bands, and most closely arranged along the margins of these pulp bands, directly under the capsule and in the neighborhood of the large vessels. As a direct result of this arrangement we have a network of pigmented cells, as shown in Plate XI, Fig. 2.

As to the exact nature of these cells we cannot be quite certain; but since our observations agree with those of Müller, who finds no definite endothelial lining to the capillary blood spaces, it seems probable that these cells, which themselves form the limits of the blood spaces, are to be considered as essentially endothelial in nature. Their form, when they have acted as phagocytes, is very similar to that of the macrophages seen in the capillaries and larger vessels of the spleen, liver and other organs. Distended sometimes to a great size and crowded with pigment balls, pigment granules, infected red corpuscles and debris of various sorts, their nuclei swell and become pale, while the protoplasm often becomes clear. Deposits of pigment also occur in the elongated cells which outline the spherules, as a result of which they sometimes become greatly distended.

Degenerative changes have also been observed in the spleen. Those occurring in connection with the bacterial infection in case No. 101 are identical in character with the necroses observed in the liver of that case.

In two cases (Nos. 103 and 111) a considerable fragmentation of nuclei was noted, more especially in the lymphoid spherules, where the bulk of the nuclei are represented by very deeply staining dots. The cells have lost their contour and the whole spherule has a degenerated appearance.

Quite a different picture is presented in the spleens of Nos. 5, 80, 31, and some others where there are well-defined annular hyaline deposits which stain bright pink. These rings often enclose groups of large macrophages containing yellow pigment. In the cases of proteosoma infection there are found also in the spleen the peculiar ovoid bodies referred to above in the description of the liver, although none of the above lesions can be definitely ascribed to their presence. These present the same appearance as in the liver, excepting that they are more frequently arranged in beautiful symmetrical groups as in a segmenting organism. These groups vary much in size, the segments numbering from 5 to 20 or more; as in the liver, they occur within large cells, which here may be definitely identified as cells of the splenic pulp. They are most numerous in the neighborhood of the ellipsoids and generally in the less richly vascular portions of the spleen. In case No. 43 there are areas in which almost every cell contains a group, some cells containing even two. It is not improbable that some light upon the nature of these bodies may be obtained later by aspirating the spleen of infected birds during life.

Bizzozero (5) has given a very good account of the structure of the *bone-marrow* in birds. It is unnecessary, however, at the present time to review his work. All writers upon the subject who have so far referred to the pigmentation of the organs in birds, have especially noted the dark color of the bone-marrow. Danilewsky (3) describes large protoplasmic bodies containing pigment and fragmented red corpuscles, and again (9) numerous " macro-melanophages." Labbé (1) and Kruse (2) find pigment in the capillaries of the bone-marrow in virulent cases.

Our own experience has not impressed us with the intensity of the pigmentation in the bone-marrow. Unfortunately, in case No. 101, where there was a most intense infection and where the other organs were deeply pigmented, the bone-marrow was lost; but in the succeeding cases where it was carefully studied no accumulation of pigment was observed, and but few organisms as such were found in the capillaries. In case No. 3 numerous organisms were seen, but no pigment blocks, and it was only in Nos. 81 and 31, where smears were made from the bone-marrow, that large pigmented cells were found in moderate numbers. These with their contents were in most respects similar to the large phagocytic cells seen elsewhere, with the exception that the pigment was in smaller masses.

But little remains to be said of the *visceral changes.* The brain in all our cases was quite normal; of course intra- and extra-corpuscular organisms were occasionally seen in the capillaries, but they were never collected in masses sufficient to occlude the lumen of the vessel. There was no pigmentation of the tissues except that a few doubtful pigment granules were situated at the poles of the nuclei of some of the endothelial cells in the capillaries in case No. 31.

Organisms were found in the capillaries of the lungs, but beyond inspired carbon particles no further pigmentation of the lung was to be made out. This is contrary to the experience of Kruse (2), who always found pigmentation of the lungs. This coal pigment is of a dead black color, readily distinguishable from either of the forms of pigment described as the result of the infection. Chemically it is not acted upon by any of the reagents employed to demonstrate the presence of iron. It is distinguishable in the neighborhood of the bronchi and air sacs and is evidently in close relation to the lymphatics.

The stomach (proventriculus) and intestine were quite normal in all the cases with the exception of the presence of a few organisms in the vessels of the mucosa.

The kidneys showed occasionally a few intravascular organisms, but were otherwise normal.

The pancreas, thyroid, heart and muscles showed nothing abnormal.

Phagocytosis, which apparently bears a close relation to all this pigmentation of the organs, has not been very satisfactorily studied in the blood. Labbé (1) denies the existence of an active protective phagocytosis; wherever he found infected corpuscles within the leucocyte the normal elements were associated with them. He admits, however, that parasites are engulfed and destroyed by the phagocytes and more especially by those of the hæmopoietic organs.

Kruse (2) has observed the engulfing by the leucocytes of the pigment left by the degenerated flagellated forms.

Danilewsky (3) followed carefully the changes seen in the infected red corpuscles on being engulfed by an active phagocyte. He finds that the stroma of the red element is first slowly digested away, leaving the intact organism, and that the organism then loses its sharpness of contour and is finally lost to view, leaving only the pigment.

The morphology of the leucocytes as given by Bizzozero (4) is briefly as follows:

(1) Cells 6.5 to 7.8μ in diameter with rather large polymorphous nucleus and clear protoplasm, in which are contained numerous highly refractive fusiform rodlets which take on an eosin stain.

(2) Cells of the same size with similar nucleus and protoplasm, but with small spherical eosinophilic granules—cells almost like human eosinophiles.

(3) Small leucocytes with large spherical nucleus and homogeneous protoplasm.

(4) Large leucocytes 6.7 to 8.3μ in diameter, spherical or oval, with large nucleus and opaque finely granular protoplasm.

Of these we have found that the first are the more common, corresponding apparently in numbers to the polymorphonuclear neutrophiles in human blood. For the sake of brevity in description it has seemed to us advisable to adopt some special term to distinguish this variety of cell. The greater richness with which these particular granules stain with acid fuchsin has suggested to us that the name *rubinophile* might not be unfitting, and it is by this term that we shall hereafter refer to them. They are evidently the chief agents concerned in phagocytosis in the circulating blood. After watching for a long time, we have observed that they sometimes engulf degenerated organisms and present then the same appearance as the pigmented leucocytes in human blood. In the fresh slide we have several times observed the leucocyte, after partly surrounding the organism, to leave it in a still further disintegrated condition and wander away. It appeared to engulf the protoplasmic remains and leave the pigment free in the plasma. Even in such an intense infection as that which had occurred in the owl (No. 101), where several organisms could be seen in every field, we found no pigmented leucocytes in the fresh blood; nor have we met with pigmented rubinophilic leucocytes in the vessels of the tissues except in one or two instances, although in clots of blood taken from the aorta we have occasionally found macrophages loaded with pigment and the detritus of organisms. The exceptions referred to were demonstrated in the liver of case No. 101 by staining with acid fuchsin. In these specimens two rubinophilic cells very much swollen and containing masses of pigment were found in the capillaries.

In these changes there is seen a marked general resemblance to those occurring in the organs of human beings suffering from malarial infection, changes which have been so accurately described by Barker (10). The chief point of difference is in the behavior of the white blood corpuscles, which appear here to play but little part in the changes in the tissues. In no instance have we observed the inclusion of the leucocyte within a macrophage and, although in the liver of case No. 81 there were great accumulations of rubinophilic cells, not one is seen to contain pigment or the remains of an engulfed parasite. The intimate nature of the different pigments here found and their relation to that found in the tissues of human beings with malarial infection merits careful chemical study.

In conclusion I wish to express my thanks to Dr. Thayer, to whom I am indebted for many kindnesses and much substantial assistance in this work, which was undertaken at his suggestion and continued under his supervision; also to Dr. Barker, who has examined my preparations and given me many valuable suggestions.

The main points in the results of the autopsies may be briefly summarized as follows:

(No. 3.) *Passer domesticus* (English sparrow); Halteridium infection.

Liver mottled, dark reddish brown; shows fatty degeneration and slight pigmentation.

Spleen greyish white; microscopically, shows few organisms and very little pigmentation.

(No. 5.) *Passer domesticus;* Halteridium infection. *Spleen* enlarged; bluish black. Microscopically, shows bands of black pigment corresponding with the pulp bands and an extensive subcapsular deposit. The dense pigment masses are intracellular. *Liver* somewhat pigmented; the large cells containing pigment lie in the capillaries. The other organs normal in appearance.

(No. 31.) *Passer domesticus;* Proteosoma infection. *Liver* large, homogeneous, dark brown. Microscopically, the capillaries of the liver are often found crowded with infected corpuscles. There are areas of focal necrosis with central hyaline deposit, loss of liver cells and many distorted nuclei (Plate XI, Fig. 1). In the capillaries surrounding these, but much oftener in the large intracapillary cells, are found ovoid bodies which have been described above. *Spleen* enlarged, black; much pigment on teasing; densely pigmented in a characteristic way and presenting annular areas of hyaline change. Numerous groups of the ovoid bodies may be found here also.

(No. 43.) *Passer domesticus;* Proteosoma infection. *Liver* large. *Spleen* 20 x 3 mm.; black. There is extensive pigmentation of both liver and spleen, and great numbers of the above-mentioned ovoid bodies are found in both organs (Plate X, Figs. 3 and 4). Post-mortem changes have masked the effects on the tissues.

(No. 80.) *Agelaius phoeniceus* (blackbird). *Liver* dark in color; slightly pigmented. *Spleen* enlarged, black. There is moderate pigmentation and very numerous regular symmetrical groups of ovoid bodies are to be seen.

(No. 81.) *Passer domesticus;* Halteridium infection. *Liver* light brownish red. Microscopically shows areas crowded with leucocytes and round granular eosinophiles; pigmentation slight. *Spleen* greyish brown; not enlarged; some pigment seen on teasing. Much pigment in bone-marrow.

(No. 101.) *Bubo virginianus* (horned owl); Halteridium infection. Bird lived some time with a broken wing and with shot in its lungs and other organs. *Liver* chocolate brown; microscopically deeply pigmented; capillaries contain numerous macrophages and other phagocytic cells. *Spleen,* 1.5 x .7 cm. (twice normal size); microscopically shows most intense pigmentation corresponding to the margins of the pulp bands, so that on section it presents a coarse network of black lines (Plate XI, Fig. 2). Bone-marrow unfortunately not preserved. In all the organs there are focal necroses in which are seen masses of bacilli of a rod-like form, staining weakly and occurring sometimes in threads. They are also found distributed in the capillaries and unassociated with necroses. Cultures were not made. This septicæmia was probably a terminal infection.

(No. 103.) *Corvus americanus* (crow); Halteridium infection. *Spleen* dark red; microscopically shows areas of cellular degeneration with fragmentation of the nuclei. These are limited to the spherules of lymphoid tissue. *Liver* normal in size and color; pigmentation not extensive; a few rubinophilic leucocytes are seen to contain pigment in the liver capillaries.

(No. 104.) *Passer domesticus;* Proteosoma infection. *Liver* brownish; microscopically, definite focal necrosis associated with the ovoid bodies, as in case No. 31. The pigment is in small dense masses and moderate in amount. *Spleen* large; 10 x 2 mm.; bluish red in color; ovoid bodies similar to those in the liver are to be found also in the spleen.

(No. 111.) *Corvus americanus;* Halteridium infection. Organs looked fairly normal. *Spleen* rather dark in color; microscopically, pigment is not abundant and has the usual distribution; occasional areas of degeneration in the lymphoid nodules. The liver cells, as in No. 103, are vacuolated and contain much fine yellowish pigment, which gives the iron reaction. Bone-marrow dark reddish brown; no pigment masses and very few organisms are to be seen.

(No. 119.) *Corvus americanus;* Halteridium infection. *Spleen* blackened; organs otherwise of normal appearance. *Liver* shows slight pigmentation; few macrophages and endothelial cells containing masses of pigment. Bone-marrow negative. There was here, as in No. 111, a coincident infection with filaria.

DESCRIPTION OF PLATES X AND XI.

PLATE X.

Fig. 1.—Portion of the liver of an infected bird showing a pigmented endothelial cell.

Fig. 2.—Macrophages loaded with pigment, etc., as seen in the spleen and liver.

Fig. 3.—From the liver of case 43, showing the intracellular ovoid bodies described p. 107.

Fig. 4.—An isolated liver cell containing a group of the ovoid bodies.

PLATE XI.

Fig. 1.—From the liver of case 31, showing a focal necrosis with loss of liver cells, which are replaced by hyaline material. In many places groups of the ovoid bodies can be seen in the necrotic tissue.

Fig. 2.—From the spleen of case 101, showing the distribution of the pigment. Focal necroses caused by bacteria are also seen in the pulp.

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(2) Kruse.—Virchow's *Archiv*, cxx, 121.

(3) Danilewsky.—Contribution à l'étude des phagocytes. *Ann. de l'Inst. Pasteur,* iv (1890), 432.

(4) Danilewsky.—Développement des parasites malariques dans les leucocytes des oiseaux. Ibid., 427.

(5) Bizzozero.—Structure de la moelle de l'os. *Arch. Ital. de biol.,* xiv (1891).

(6) Wilh. Müller.—Ueber den feineren Bau der Milz, 1865.

(7) A. J. Whiting.—Spleen. *Proc. Edin. Roy. Soc.*, 1896.

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(9) Danilewsky.—Zur Lehre von der Malaria-Infection bei Menschen u. Vögeln. *Arch. f. Hygiene,* xxv (1895), 227.

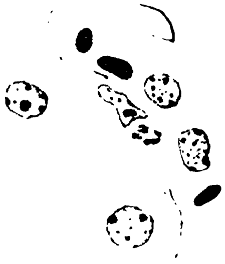
(10) Barker.—Study of some fatal cases of malaria. *Johns Hopkins Hospital Reports,* v, 1895.

(11) Danilewsky.—Parasitologie comparée du sang. Kharkow, 1889.

(12) Ponfick. Studien über die Schicksale körniger Farbstoffe im Organismus. Virchow's *Archiv,* xlviii (1869), 1.

(13) Neumann. Das melanämische Pigment. Virchow's *Archiv,* cxvi (1889), 318.

(14) Quincke.—Ueber directe Fe-Reaction in thierischen Geweben. *Arch. f. exp. Path. u. Pharm.,* xxxvii (1896), 183.



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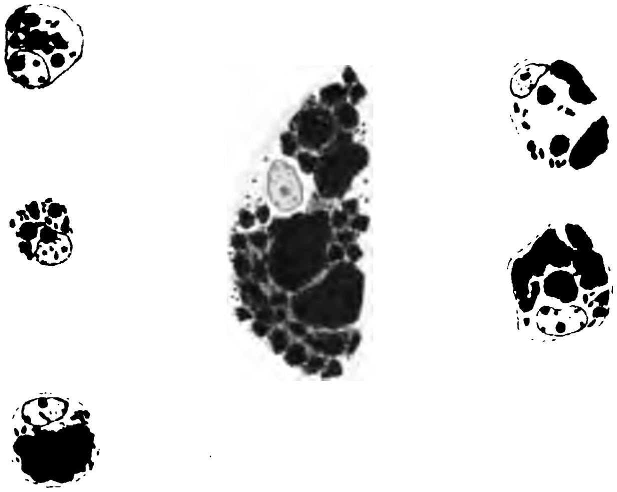

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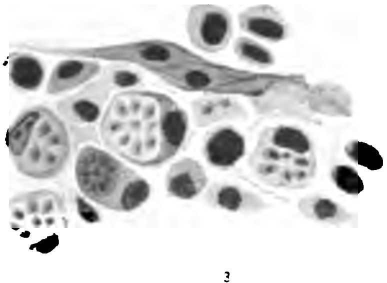

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PLATE XI.

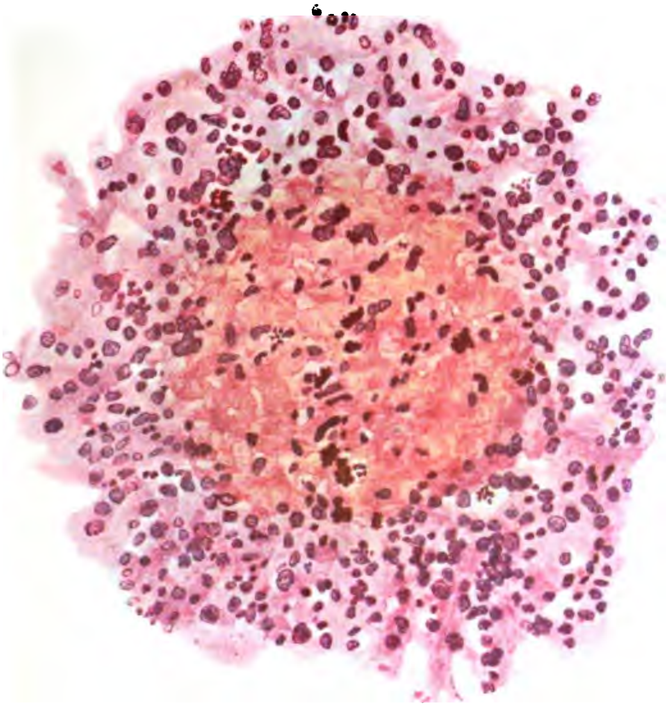

Fig. 1

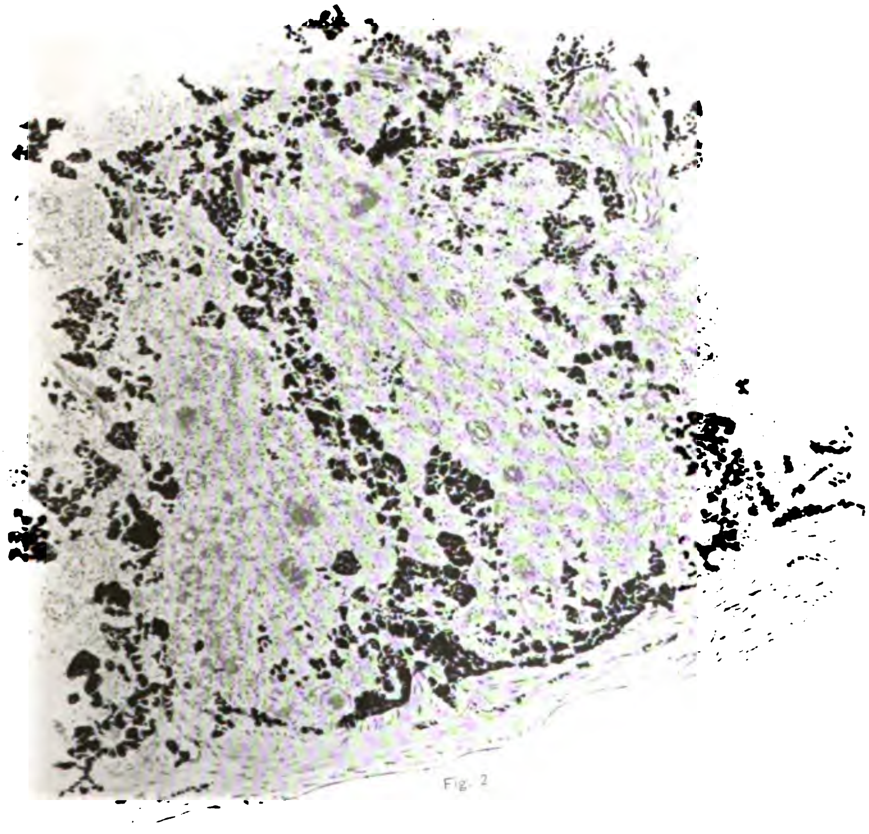

Fig. 2

ON THE HÆMATOZOAN INFECTIONS OF BIRDS.

BY W. G. MACCALLUM, M. D. (JOHNS HOPKINS UNIVERSITY).

PLATE XII.

Our knowledge of the nature of these infections, sometimes loosely designated as avian malaria, is mainly due to the researches of Danilewsky, Laveran, Kruse, Sakharoff, Grassi and Feletti, Celli and Sanfelice, Pfeiffer, Di Mattei, and Labbé. The only work upon this subject so far done in America was reported in the papers on the etiology and pathology of hæmatozoan infections in American birds, read by Opie and myself before the Medical Society of the Johns Hopkins Hospital in November, 1896, and printed in brief in the *Bulletin of the Johns Hopkins Hospital* for March, 1897. These papers, which are published in full in the present number of this Journal (pp. 79 and 103), give the results of observations made at the suggestion of Dr. Thayer of the Johns Hopkins Hospital, in the summer of 1896, on the blood and organs of various birds.

During the past summer (1897) I have continued the work in Dunnville, Ontario, confining my attention mainly to crows, which seem to afford the most promising field for observation.

Many species of birds have been found to be infected with these organisms to a greater or less degree—in Europe sparrows, finches, starlings, blackbirds, crows, hawks, etc. In Ontario and Maryland, where we have procured our birds, we have found sparrows, blackbirds, owls and crows infected, and of these the crows were most frequently so, for although the number is too small to furnish any definite conclusions as to relative frequency of infection, some idea of this may be gained from the fact that of eighty sparrows the blood of five showed organisms, while of twenty-one crows sixteen were infected.

The organisms in these cases are very similar in many respects to those found in the blood of human beings suffering from malaria.

There are, however, certain well-marked differences which I shall briefly review before detailing the results of my work of the past summer. According to the observations of Labbé,* and those of Opie,† two types of organisms may be distinguished differing in form and life history as well as in the effects produced on the host. Of these the first, named by Labbé "Halteridium," begins its cycle of existence as a small oval or rounded hyaline body, situated laterally or at one end of the nucleus of the red corpuscle. As it increases in size it extends in a direction conditioned by the position of the nucleus, at the same time by its metabolic activity forming in itself granules of blackish-brown pigment. As it grows it extends more and more along the sides of the elliptical nucleus until finally, on reaching its adult stage, it is seen to coil about the nucleus, embracing it quite closely and expanding at each end into a wider portion into which the pigment, which increases with the age of the organism, is often gathered. Stained specimens show a tingeable cytoplasm in which the pigment is embedded and within which lies a clear non-stainable body with a central stained spot. This clear body is thought to be the nucleus with central nucleolus, and plays an important part in the process of segmentation as described by Labbé. He finds that the nucleus of the parasite divides into two segments, which separate and proceed to the ends of the organism, where they divide into multiples of two, thus forming two rosettes of segments lying at the ends of the red corpuscle. In the middle of the red corpuscles beside the nucleus there remains the pigment together with some debris of the organism. Opie has not been able to confirm this observation, but has seen the formation of single rosettes in the bone-marrow. I have also found forms which might be designated as pre-segmenting in the circulating blood. In these the organism had rounded itself off in one end of the corpuscle, the pigment being gathered into a mass in its centre, the margin showing a number of indentations corresponding with which there were the fine refractive dots thought to represent the nuclear flecks in each segment.

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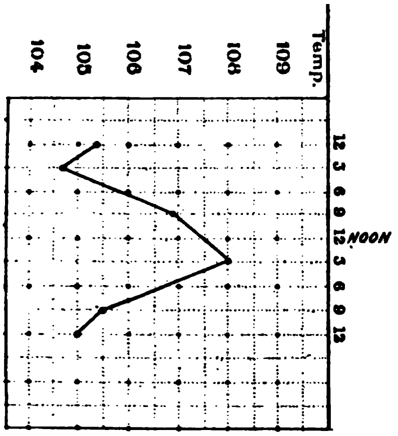
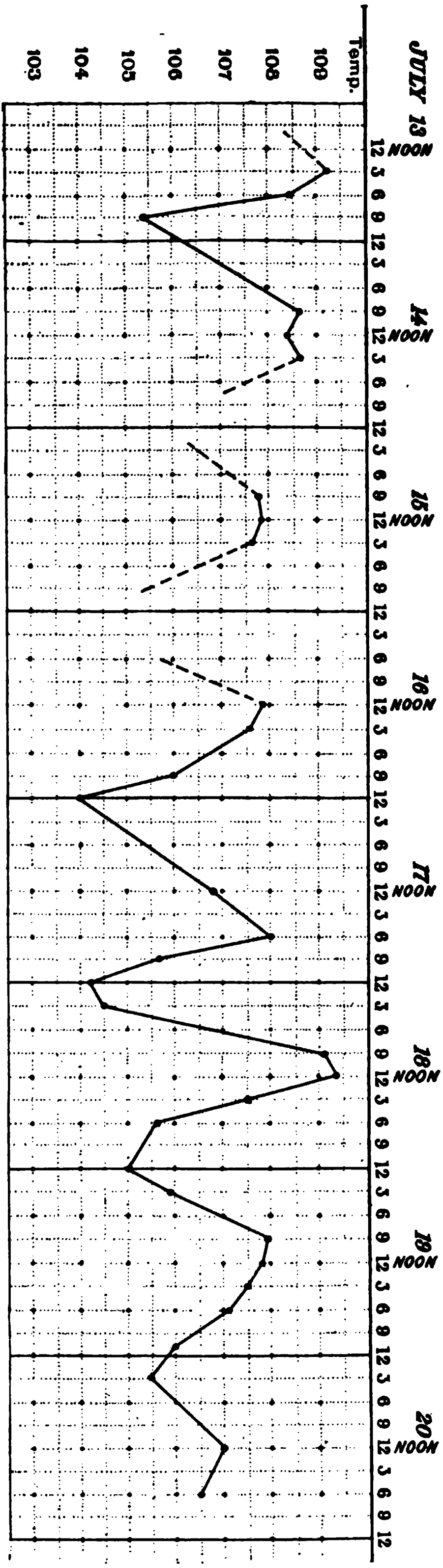
* *Arch. de zool. expér.*, série 2, 1894.

† The present number of this Journal, p. 79.

Chart I, showing Daily Temperature of a Normal Crow.



Temperature Chart II of Crow infected with Halteridium.*



Flagellation almost identical with that observed in the case of the human malarial organisms is very readily seen in these organisms. The exact length of the cycle is very difficult to determine, because all the organisms present in the blood of any bird do not segment at the same time, nor even, so far as can be determined, in large groups, but at any time almost all stages can be seen. Labbé places the cycle at seven to eight days. He, as well as Danilewsky,† states that very little can be observed clinically in infections with this form of organism, although in the case of the second form about to be described, there are febrile symptoms with a certain rise in temperature. I have attempted to construct the accompanying chart from a series of observations on the temperature of a crow infected with the Halteridium and, although it does not extend over any great length of time, it will serve to give some idea of the course of the temperature as compared with that of a crow which showed no infection. There is seen to be a daily rise of from 3 to 5 degrees from the temperature recorded at midnight, and this, to a certain extent, is true of the normal crow, so that it can hardly be ascribed to the effect of the organisms, but we must perhaps rather consider the crow to be, to a certain extent, poikilothermic.

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*Kindly charted for me by Miss Hutchinson of the Training School for Nurses of the Johns Hopkins Hospital.

†*Arch. f. Hyg.*, xxv (1895), 227.

There is, however, a rather higher temperature recorded on July 13, 18, etc., suggesting that some group of considerable size might be segmented at intervals of five days. This, however, is not very constant and may have been accidental, although the blood on these days of high temperature seemed to show a larger percentage of hyaline forms.

A variety of this Halteridium is found in the crow, blackbird, etc., in which the organism is much more slender, and when full-grown finds room to lie in the corpuscle without touching the nucleus.

The second type of organism, named by Labbé "Proteosoma," also begins its cycle as a small, rounded or ovoid, hyaline body, generally situated near one end of the red corpuscle. As it grows, instead of accommodating itself to the position of the nucleus, it causes such a change in the stroma of the corpuscle that the nucleus no longer retains its central position, but is pushed to the opposite end of the corpuscle, where it comes to lie transversely. The pigment, which is on the whole finer and blacker than that of the preceding form, makes its appearance early and is distributed over the body of the parasite, which, in its adult stages, takes on a rounded or irregular polygonal form. Flagellation occurs precisely as in Halteridium. Segmentation takes place in the circulating blood and results in the formation of a simple rosette with a central mass of pigment. As stated above, this form of infection seems to be accompanied by more serious clinical phenomena than the other.

The Proteosoma I had no opportunity of observing during the past summer, and it is more especially of the Halteridium, which occurs so abundantly in crows, that I here wish to speak. The infections with the latter organism are generally relatively slight—one or two organisms being found in a slide of blood—and the birds may show no external evidence of being sick. There are other cases, however, where the bird can be recognized at a distance as being sick by its evident weakness, its uncertain flight and altered voice, as well as its draggled appearance and lustreless plumage. Such birds often show an enormous infection, at times more than a third of the corpuscles being invaded, with sometimes two or even three parasites in a single corpuscle. They may, however, recover to a certain extent in confinement when well fed, and the organisms may be seen to diminish in number most markedly.

On examining a slide of fresh blood where the organisms are numerous, examples of flagellating forms are observed with extreme readiness about two and a half minutes after the blood has been taken from the bird (Plate XII, Fig. 6). This process, accurately described by most authors on the subject, takes place as follows: The adult organism is seen to draw itself together into a sphere within the red corpuscle, and sometimes immediately, but more often after a short delay, it begins to be greatly agitated, the pigment dancing about and the surface of the sphere taking on an active undulating motion which lasts but a short time, for the organism suddenly bursts from the corpuscle, scattering the remains of the latter, and in its place beside the nucleus of the corpuscle, which now lies free in the plasma, it throws out four or more flagella, which thrash about wildly and sooner or later become detached and wriggle away. The sphere is much reduced in size by this throwing out of flagella and the pigment is concentrated, although occasionally a pigment grain is included in one of the flagella, which are not perfectly regular in form, but may branch or run into one another at the base. Their number is not constant and may change in the process of their formation. The remains of the sphere continue to be agitated and, after the loss of the flagella, its pigment sets up a most active dancing. Often it constricts itself into two or more parts, which may reunite, and sometimes a more than usually stout prolongation of protoplasm, which has failed to become a perfect flagellum, may adhere and beat about for a long time. Disintegration and death are the inevitable fate of these remains of the flagellate body, even if it escapes for any length of time one of the voracious leucocytes which wander about.

There is a certain variation in the relation of the act of throwing out flagella to the extrusion from the corpuscle. At times the organism can be seen to throw out flagella while still within the corpuscle, these beating the nucleus about within the enveloping membrane of the corpuscle, which is violently agitated and distorted like a loose bag containing the struggling organism. At last the membrane bursting liberates the fully developed flagellate form. In other cases the sphere slowly escapes through a rent in the capsule, and after a period of quiescence outside the corpuscle, begins to be agitated and finally to put forth its flagella.

Other organisms escaping quietly in this way through a rent in the capsule remain quiescent and never become flagellated (Plate XII, Fig. 5).

There is always in these processes the escape of the remaining hæmoglobin of the corpuscle into the plasma, with a corresponding blanching of the corpuscle and, although the thin capsule is left for a time, it soon becomes invisible.

Many theories as to the nature of these flagellated forms and the significance of the process have been put forward, none of which have met with general credence. Laveran thought the flagella were preexistent in the body of the intracorpuscular parasite. He believed the flagellate forms of the human malarial organism to be the final and most perfect stage of the parasite, and that they developed within the round and crescentic bodies which he regarded as cysts. Dock considered that they represented a resting stage of the organism capable of existing independently, perhaps even of reproducing themselves, but also capable under favorable circumstances of reproducing a typical growth of the parasite. Mannaberg suggests that they are organs which permit the parasite to enter into a saprophytic existence. "I suspect," he says, "that the flagellate bodies enter upon the first stages of a cycle of existence outside of the human body, and that as a result of the unfit culture medium the death of the young spores occurs." The whole Italian school believes them to be due to degenerative changes. Manson, as is well known, has advanced the idea that the flagellate bodies represent the forms in which the parasite exists outside the human body, that the flagella penetrate from the stomach into the body of mosquitos which have sucked the blood of infected human beings, and that, after a further unknown process of development, they come again, through the water in which the mosquitos deposit their eggs and die, into the human body. Sakharoff recognizes the flagellate bodies as dying forms; he finds the large red nucleus (with Romanowsky's stain) with a clustered chromatic filament. In the free forms this last breaks up into several filaments, which leave the parasite as flagella—a perversion of karyokinesis which is provoked by physico-chemical means. Zacharias claims to have produced flagella in epithelial cells by the action of phosphoric acid, and Labbé gives it as his opinion that the process of flagellation resembles a chemical one, that lack of oxygen hastens the process, as also does the sudden drop in temperature from 42 to 15 degrees C. Flagellate forms, he concludes, are abnormal agony products preceding degeneration, which do not exist in the living blood, but form outside the body under the diverse physico-chemical influences which modify the plasma and corpuscles obtained from the vessels.

It will be seen that none of these ideas, except that of Sakharoff, are based on any observations, but are for the most part pure hypotheses. That the flagellate forms are the agony products of the dying parasites seems to be the most generally accepted idea, and this, it is true, has the fact to support it that it occurs only under unfavorable circumstances, and is followed by the death of the organisms.

Other motile bodies have from time to time been described as occurring in the blood of birds. Danilewsky * describes elongated non-pigmented organisms found in fresh blood plasma. These are seen to swell and contract and frequently to advance in a curved or spiral line. He thinks they are related to the Drepanidia, but Labbé suggests with more probability that they are free flagella.

In addition to these Danilewsky also describes an organism resulting from the transformation of an intra-corpuscular pigmented form. This becomes extra-corpuscular and spherical and, after the lapse of ten to thirty minutes, a process appears at the periphery of the body which, increasing in size, gives rise to a curved elongated body which he calls a vermiculus. This body, which has acquired a nucleus,

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* Danilewsky, Parasitologie comparée du sang. Kharkow, 1889.

moves about in a curved line in the plasma. Kruse * described a similar form in the blood of a crow, and Pfeiffer † in that of an owl. No further idea as to their nature was given.

It was noted by Opie (p. 92) that there were variations in the adult form of the Halteridium, which I have endeavored to depict in Plate XII, Figs. 1 and 2 and Figs. 3 and 4. This difference, which sharply distinguished two forms of the organism, consisted in a granular appearance in the one as compared with the clear hyaline protoplasm of the other forms. The protoplasm in the first form was not simply finely granular, but in a groundwork of finely granular protoplasm could be seen minute hyaline points. This difference was much more sharply brought out by staining with methylene blue when the granular form took on a deep stain, the fine granules especially appearing dark blue while the hyaline points remain wholly unstained. The hyaline form, on the other hand, took almost no stain, the slight tinge of blue being for the most part peripheral, sometimes with a blue fleck in the centre of the unstained portion. The pigment granules are, as a rule, finer in the granular forms than in the clear hyaline forms.

Opie (p. 96) put forward the suggestion that it might be these clear non-staining forms which become flagellated, the granular forms being the ones observed to degenerate without flagellation.

My observations have enabled me to confirm this distinction, and to demonstrate that it is true that the granular forms never become flagellated and that the clear forms alone do so. Indeed, it may be prophesied of any organism while yet intra-corpuscular that it will or will not become flagellated.

While watching one of these specimens which had stood for some time I was surprised to see an elongated organism moving slowly across the field followed by others moving in all directions. These were evidently modifications of the organism familiar in fresh specimens, and the question of greatest interest was to show their relation to the other forms. They corresponded very closely with the vermi-

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* Kruse, Virchow's *Archiv*, cxx (1890), 541, and cxxi, 359.

† Pfeiffer, *Zeitschr. f. Hyg.*, viii (1890), 309.

culi of Danilewsky, and I have assured myself since that they are identical with them.

As the forms which flagellated were always observed to disintegrate or succumb to a leucocyte, the idea remained that the motile forms might arise from the forms described as being extruded from the corpuscle without flagellation. On carefully watching these for fifteen to twenty minutes it proved to be easy to see the change from the non-motile sphere into the motile elongated organism or vermiculus. In one or two instances, however, it was noted that such non-motile spheres apparently became flagellated without the extreme agitation usually seen in the process of flagellation, and without any diminution in their bulk, and later on gave rise to the motile forms. This seemed to contradict the idea that these arose exclusively from the non-flagellating forms, and apparently there remained the conclusion that flagellation was a process which proceeded more or less violently in different cases; that where the violence was great the store of energy of the organism was exhausted; where, however, it did not take place, or took place in such a modified and mild form that not much energy was expended, the organism was able to take on a motile form.

I decided, however, on the impulse of another idea, to observe carefully in the same field a granular form and a hyaline form from the time of extrusion from the corpuscle to the beginning of the motile stage, and, having found such a field, the following picture presented itself: The two forms lay at some distance from one another separated by the plasma and a few corpuscles. The granular form happened to escape from the corpuscle first and lay perfectly quiet beside the free nucleus and the shadow of the corpuscle. Soon the hyaline body, becoming greatly agitated, burst from the corpuscle and threw out active flagella, which beat about for a few moments and finally tore themselves loose. Then came the acme of the process. One of the four flagella passed out of the field, but the remaining three proceeded directly toward the granular form, lying quietly across the field, and surrounded it, wriggling about actively. *One of the flagella, concentrating its protoplasm at one end, dashed into the granular sphere,* *which seemed to put out a process to meet it, and buried its head, finally wriggling its whole body into the organism, which again became perfectly round.* The remaining flagella, seeking to repeat this process, were evidently repulsed, and soon became inactive and degenerated. Immediately on the entrance of the flagellum the pigment of the organism was violently agitated, without, however, any disturbance of the outline of the organism. Soon all became quiet again and the period of quiescence lasted for about fifteen minutes, when a conical process began to appear at one margin of the organism, which, increasing in size, drew into itself most of the protoplasm, the pigment to a certain extent being gathered into the remainder. Finally most of the pigment was concentrated into a small round appendage, which remained attached to the end of what now had became an elongated fusiform body, which soon swam away with a gliding motion.

Have we not here, without much doubt, a sexual process in the organisms the result of which is the motile vermiculus? This is a process which we might have expected and which I am confident will be found to occur in the case of the human malarial parasites, although on account of the small number of organisms to be found in each slide in the latter case, the completion of the process will rarely be observed. It is entirely analogous to the sexual process as it occurs in the lower plants under unfavorable conditions. In Spirogyra and other related algæ, when their circumstances have become such that the ordinary reproduction by fission can no longer result in the successful prolongation of life, a more resistant body is formed by the conjugation of the adjacent cells of two filaments; but so long as conditions of growth are favorable we look in vain for this process. A similar conjugation is observed in many of the Ascomycete fungi and many other plants. In the animal kingdom we find it again and again, and I need only mention the pond Amœba as a familiar example.*

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* Since the communication of this paper to the British Association for the Advancement of Science in Toronto on August 24, 1897, I have been much interested in the perusal of the article by Simond (*Annales de l'Institut Pasteur*, July 25, 1897, p. 545), in which he describes in certain coccidia a process analogous to that described here and suggests the probability of the occurrence of a sexual process in the malarial organism.

We can thus consider the two forms of adult organism found in the fresh blood as male and female, the granular female form containing more chromatophilic substance than the hyaline male form, which we may now say gives up its life in the production of four or more spermatozoa or flagella. Now, too, the mistake of thinking that the motile forms might arise from organisms in which the process of flagellation had proceeded less violently, is easily explained. Such organisms were simply the spherical granular forms surrounded by spermatozoa or flagella, which had arrived from the neighboring true flagellate forms and were undergoing the process of fertilization.

In view of all this it seems to me that we must look on the observations of Sakharoff with more confidence than has been accorded them by authors in general. Although I have stained with an excess of gentian violet the free flagella in the plasma and surrounding female spheres, I have not been able so far to confirm his statements by the application of staining reagents, but I feel assured that, were such nuclear staining successfully carried out, we should see in all essentials his clustered chromatic filament which, on breaking up into several filaments, forms, each with a certain amount of protoplasm, several flagella. His ideas as to the cause of the process are rather more hypothetical.

To return to the organism, immediately after fertilization, a process which when once recognized I have observed daily, it seems clear that the agitation of the pigment on the entrance of the flagellum is due merely to the mechanical motion of the wriggling flagellum, for it quiets down very soon. From this point on for ten to fifteen minutes it seems probable that, with suitable nuclear stains, one might demonstrate the coalescence of male and female nuclei, but I have not as yet done so. After a lapse of fifteen to twenty minutes the organism becomes motile, as above described, by the passage of the protoplasm into a conical process (Plate XII, Fig. 8), which gradually enlarges so as to contain the whole organism with the exception of a small spherical appendage into which most of the pigment is crowded (Plate XII, Fig. 9). The organism now appears quite fusiform, or at times egg-shaped, or even almost spherical, the anterior small end being free from the pigment, which is distributed quite superficially over the posterior part. Toward the anterior part, but sometimes quite in the middle of the organism, there lies a fairly definite nucleus with well-marked nucleolus. This nucleus does not stain sharply, but is quite readily seen in fresh specimens, although no structure so definitely marked off by its different refractive index was observable in the adult male and female forms of the organism. The protoplasm retains the characteristic granular appearance seen in the female form.

Occasionally in beginning its movements away from the nucleus of the red corpuscle the organism leaves behind it part of the pigmented appendage—or it may possess two of these spherical appendages attached one behind the other, and in starting leave one of these behind to be broken down or engulfed by a leucocyte. The movement has nothing of the character of that of the flagellate forms, but is slow and even, the organism progressing invariably with the pointed end, free from pigment, forward. It can move in any direction readily, curving about obstacles and passing accurately between groups of corpuscles. Often it is seen to rotate continually on its long axis, at which times the superficial position of the pigment is readily seen. This rotation does not seem, however, to be in any way necessary to the forward motion. Again a third form of motion is observed in that a sort of peristaltic wavy constriction may repeatedly pass from anterior to posterior end of the organism, giving rise to very considerable distortions.

The forward progression of the organism occurs with very considerable force, as one may often be seen to thrust aside a group of red corpuscles. More often, however, they push directly through the obstacle, and it is interesting to observe that they can and do dash through the body of a leucocyte, tearing it apart, scattering its refractive granules into the plasma and often dividing its nucleus into two parts, thus leaving it to disintegrate. Passing on unharmed they attack masses of red corpuscles and leave those in their path as shadows of which soon only the nucleus is visible. This blanching of the corpuscles seems to become possible to them by the merest touch of their anterior pointed ends, which apparently puncture the capsule of the corpuscle and allow the hæmoglobin to escape into the plasma. Indeed, in a thick slide of blood, a single organism may circle about and clear for itself a very considerable space in which there remain only shadows with the nuclei of corpuscles, and in some of the intense infections, which I have referred to, every slide of blood, after standing from thirty minutes to an hour, will show nothing but shadows, the organisms, very young and half-grown forms as well as segmenters, being often left free in the plasma. On one or two occasions these forms have been seen to put out pseudopods which attacked the corpuscles in a similar way.

In passing through a group of corpuscles it is frequently observed that the posterior end of the moving organism adheres to the corpuscles, and it may often be seen dragging three or four behind it. These soon become detached, however, and fall back, sometimes greatly distorted. In other cases the organism adhering to the corpuscle may draw out its capsule into a process, which gradually sinks back when the organism breaks away.

The ultimate fate and the true significance of these motile forms is difficult to determine, for in a slide, after being watched for more than one or two hours, the conditions become such that conclusions can scarcely be drawn as to the result under less artificial circumstances. Simple quieting down and final disintegration seem to be the end of many, but in some cases the organism was seen suddenly to stretch itself out by jerks into a greatly elongated form, sometimes dividing into segments connected by a fine thread. Generally in such cases the clear anterior end became separated from or connected only by a thread with the rest (Plate XII, Fig. 10), and often finally swam away by itself, leaving the rest to the leucocytes. This clear portion, however, was never seen to accomplish anything other than disintegration, although it was often watched with the idea that it might enter a corpuscle and set up a new cycle of existence.

It seems to me most reasonable to suppose that the motile form is the much sought resistant stage, or perhaps the first step in the production of such a stage. It seems probable that by the aid of such powerful means of locomotion and penetration it might make its way through a considerable obstacle of tissue to gain a new medium. This of course might occur in several ways in the dead body subject to external violence. But in no case which I have examined post mortem did the organisms show any signs of life after the lapse of two to three hours, although the blood was still perfectly fluid in the vessels. In the living body, however, it seems that the intestine would form the most ready means of escape, and accordingly search was made in the fæces for such forms. In the birds at my disposal, however, in some cases the infection was so slight that results could hardly be expected, and in the cases where the infection was large, the presence of numerous echinostomes in the rectum rendered the mode of entry of the abundant blood corpuscles and organisms into the intestine doubtful. In dried smears from the contents of the small intestine, however, free organisms, free malarial pigment and one intra-corpuscular organism were found. More certain data might be expected from sections of the intestine with its contents, and examination of such sections showed immense numbers of organisms, mostly intra-corpuscular but sometimes extruded, in the capillaries of the stroma of the intestinal mucosa. In the mucous contents of the intestine were numerous *free* organisms, several of which exhibited the form described above as characteristic of the motile stage. The fact that the organisms were lying quite free in mucus, and that no corpuscles or nuclei of corpuscles were found, argues in favor of the idea that these organisms penetrated the mucosa after extrusion from the corpuscle. No organisms were found on the way through the epithelium. The inference that the organisms escape thus from the body seems plain, and further examinations of the intestinal contents obtained fresh immediately after death would be interesting.

In concluding this portion of the paper I may be allowed briefly to summarize what I have said.

Two types of organism occur so far as is known in the blood of birds. Minute morphological descriptions of these have been omitted in this paper. In the adult stage of one of these (Halteridium) there are certain differences between two forms which are thought to be sexual differences. Of these forms, one on extrusion from the corpuscle lies quiet and is soon approached and fertilized by a "flagellum" pro-

duced by the other form on extrusion from the corpuscle. The result of this fertilization is a motile stage which seems more resistant, and it is thought possible, in view of certain facts, that this form may make its way through the intestinal canal to the external world.

Reasoning by analogy it is thought very probable that the significance of the flagella in the human organism is similar to that given here for the organism of the birds.

THE PATHOLOGY OF HAEMATOZOAN INFECTIONS OF BIRDS.

The literature on the pathology of hæmatozoan infections is very meagre indeed. Danilewsky * speaks of the changes in the spleen and bone-marrow and describes macrophages with their varied contents. Kruse † made similar observations as to the spleen, bone-marrow, liver and lung, and Labbé ‡ also speaks in a general way of the gross changes in the organs. In the preceding paper on the subject, in this Journal (p. 103), prepared a year ago, I have given an account of the microscopic appearances of the various organs. I desire here to present a general summary of the more important pathological changes based upon the former observations and those made during the past summer.

Notwithstanding what has been said to the contrary, phagocytosis goes on very actively in the blood, as seen in a fresh slide. The leucocytes are seen to be contracted into small spheres on first examining the fresh drop of blood, but after about five minutes they begin to wander about and engulf whatever is foreign to the normal blood. These few minutes of inactivity are probably due to the shock of making the preparation. Both the eosinophiles and the rubinophiles (p. 113) show themselves to be very voracious. In the hardened tissues, however, these cells, which are readily recognized by their oxyphilic granulations, are very seldom seen to contain any pigment. On the other hand the cells most actively phagocytic there are principally the endothelial and connective-tissue cells. The deposition of pigment, together with the remains of infected corpuscles, organisms, etc., forms the main pathological change in the organs, and in order to make this change plain I shall begin by describing an organ in which it is most simple.

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* *Ann. de l'Inst. Pasteur*, iv (1890), Parasitologie comparée, 1889.

† Virchow's *Archiv*, cxx and cxxi.

‡ *Arch. d. zool. expér.*, 1894.

In the *kidney*, as in all the other organs, the capillaries and blood-vessels generally show a great number of infected corpuscles and some organisms extruded from the corpuscle. The epithelium of the tubules is in general fairly well preserved, although there are numerous instances of a hyaline degeneration of the cells. The glomeruli show nothing except the organisms within their capillaries. It is in the connective-tissue skeleton of the kidney with its contained capillaries that we see the main results of the infection. Many of the endothelial cells of the capillaries are much swollen and contain masses of pigment, yellow as well as malarial. Besides these there are cells belonging to the connective-tissue framework, which have become storehouses of pigment—large cells with large round or oval vesicular nucleus, which have become so loaded with pigment, etc., as to be very conspicuous in the section. No epithelial cells have been seen invaded.

In the *intestine* the connective-tissue cells of the stroma are phagocytic and sometimes contain considerable masses of pigment. Similar cells occur also in the muscular wall imbedded among the muscle fibres, the pigmentation being deepest in the subperitoneal portion. The endothelial cells of the vessels in this tissue are also pigmented. The epithelial cells of the mucosa never show foreign contents.

In the *bone-marrow* we have, besides fat cells and blood-vessels, cells large and small, which are generally spherical, with round nucleus and containing basophilic granulations, and other rounded cells and nuclei of various sizes with homogeneous protoplasm, all supported by a loose skeleton of connective tissue, made up of irregular, sometimes branching cells with pale ovoid nucleus. Of all these varieties of cells only the last are seen to be pigmented. Other large round cells do occasionally occur in the bone-marrow, however, which correspond closely to the red-corpuscle-carrying cells of the human spleen; these often contain several red corpuscles, some of which are infected.

In the *lung*, *brain*, and *pancreas*, although the capillaries contain many organisms, hardly any pigment deposit can be seen.

The thyroid and *adrenal*, however, show a very considerable pigmentation exactly corresponding in nature with that described for the kidney, the pigment being deposited in the endothelial and connective-tissue cells of the interglandular tissue, the secreting cells escaping. The adrenal, however, differs in that red-corpuscle-carrying cells occur in its small vessels together with large free pigmented cells with hyaline protoplasm and vesicular nucleus. These resemble precisely the macrophages in the spleen, to be described presently. In the tissue adjoining the thyroid gland there are large spaces lined by epithelioid cells, many of which contain pigment masses, or even several infected corpuscles. Lying free in these spaces are many large red-corpuscle-carrying cells together with numerous red corpuscles, leucocytes and cells desquamated from the walls, surrounded by a fine network apparently of coagulated fibrin. The nature of these spaces I do not know more exactly.

In the *pectoral muscle* in one case there was a greyish opaque spot which on section showed a mass of necrotic material lying in the muscle and surrounded by richly cellular tissue, which was quite deeply pigmented. The neighboring muscle fibres were much fragmented, with a proliferation of nuclei reminding one of that seen in the muscle in cases of trichiniasis. The tissue about the necrotic material had some of the characters of granulation tissue and was quite vascular, the elongated cells with large vesicular nucleus being the pigmented ones. Scattered about in this tissue were numerous giant cells with necrotic centre and many nuclei, such as are seen in miliary tubercles. Some of these were very large and without definite boundaries. The large mass of necrotic material was closely bordered by groups of clustered nuclei, suggesting that it had arisen from the coalescence of many giant cells. Through it there was scattered a good deal of malarial pigment. Whether all this diseased tissue in the muscle was due to the action of the hæmatozoon is doubtful; it seems to me much more probable that the deposit of malarial pigment throughout it was entirely secondary to its formation by some other process.

In the *liver* we have often a very extensive deposit of pigment. The liver cells themselves are never phagocytic, but the endothelial cells of the capillaries are often greatly swollen by their load of pigment and debris. In the blood-vessels, especially the branches of the portal vein, we find lying free the large macrophages, such as were seen to occur in the adrenal. These sometimes are so large as to contain masses of pigment equalling in size three or four liver cells. There are other phagocytic cells with large irregular nucleus which seem to lie between the liver cells and the endothelium and, although at first sight they have somewhat the appearance of liver cells, I am inclined to think them analogous to Kupffer's cells.

It is in the *spleen* that these processes reach their acme. This organ is almost always greatly swollen and blackened, the deposit of pigment being so disposed as to show a black network on section. The vascular pulp of the spleen is arranged in bands forming a network in whose meshes lie the lymphoid elements. The pigment deposit is confined to these vascular bands beginning at the margins, where they are sharply marked off from the lymphoid tissue, and gradually involving most of their width. The bands appear to be made up of a spongy reticulum of endothelial cells, between which the blood flows. These cells are more closely arranged at the boundaries of the lymphoid tissue, where they become conspicuous early in the infection by their pigmentary contents. In more intense infections a great majority of the fixed cells making up the pulp become loaded with pigment, and thus give rise to the black network mentioned above. It seems probable that such loaded cells may be washed into the blood stream and constitute the so-called macrophages. These have been mentioned as occurring in the blood of the liver and adrenal, and they are also seen in numbers in the veins of the spleen. They are cells varying greatly in size but generally of a somewhat rounded outline and with one or sometimes two vesicular nuclei; their protoplasm is generally very clear and is seen to contain a great deal of foreign material—yellow masses of pigment as well as the balls, irregular masses, and granules of malarial pigment, broken-down blood corpuscles and disintegrated nuclei, together with shrunken parasites. The last sometimes occur in great numbers and form the only contents of the cell. In the spleen as elsewhere the endothelial cells of the vessel walls are often seen to be loaded with pigment. Red-corpuscle-carrying cells are not frequently seen.

The pigment found in all these cells is not exclusively that formed by the parasitic organisms, as has been mentioned. A large part of it is the iron-holding compound, or mixture of compounds, grouped under the name hæmosiderin, which are formed from the hæmoglobin set free on the rupture of the infected corpuscles.

We have, then, as the result of the hæmatozoan infections the production of one form of pigment by the parasites from the hæmoglobin, and of another form by the tissues of the host from the hæmoglobin set free into the blood. These solid pigments are stored up in the various organs, but more particularly in the spleen and liver, in cells belonging exclusively to the connective-tissue group which also engulf the infected corpuscles, broken remains of corpuscles and shrunken dead parasites. The anatomical changes, therefore, differ very slightly from those seen in the human being in malarial infections, so well described by Bignami* and by Barker,† the greatest difference perhaps lying in the less important part played by leucocytes in the case of the bird.

Finally, I wish to express my indebtedness to Dr. Thayer, of the Johns Hopkins Hospital, for his many kindnesses to me while engaged in this work, which was undertaken at his suggestion.

ADDENDUM.

Since the preparation of this article for the press I have examined the blood of a woman suffering from an infection with the æstivo-autumnal type of organism, in which a great number of crescents were to be seen. These in a freshly made slide of blood, with very few exceptions, retained their crescentic shape for only a few minutes. They soon drew themselves up, thus straightening out the curve of the crescent while shortening themselves into the well-known ovoid form.

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* Bignami, *Bull. d. r. Accad. med. di Roma*, xix (1892-3).

† Barker, *Johns Hopkins Hospital Reports*, v (1895).

After the lapse of 10 to 12 minutes most of them were quite round and extra-corpuscular, the "bib" lying beside them as a delicate circle or "shadow" of the red corpuscle.

After 20 to 25 minutes certain ones of these spherical forms became flagellated; others, and especially those in which the pigment formed a definite ring and was not diffused throughout the organism, remained quiet and did not become flagellated. In a field where an example of each form could be watched, the flagella broke from the flagellated form and struggled about among the corpuscles, finally approaching the quiet spherical form; one of them entered, agitating the pigment greatly, sometimes spinning the ring about. The rest were refused admission, but swarmed about, beating their heads against the wall of the organism. This occurred after 35 to 45 minutes.

After the entrance of the flagellum the organism again became quiet and rather swollen, but although in the two instances in which this process was traced the fertilized form was watched for a long time, no form analogous to the "vermiculus" was seen.

This process has also been observed once by Drs. Warfield and Pancoast of the Johns Hopkins Hospital in another case of infection with the æstivo-autumnal parasite. It is evidently for the human being what was foreshadowed by the organisms of the bird.

DESCRIPTION OF PLATE XII.

All of the figures are of the Halteridium form of the parasite.

Fig. 1.—Adult granular, or female form.

Fig. 2.—Adult hyaline, or male form.

Fig. 3.—Adult female preparing for extrusion from the corpuscle.

Fig. 4.—Adult male preparing for extrusion from the corpuscle.

Fig. 5.—Adult female extruded and lying beside the nucleus of the corpuscle.

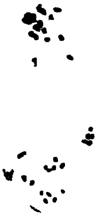
Fig. 6.—Flagellate form.

Fig. 7.—Process of fertilization.

Fig. 8.—First stage in formation of motile form.

Fig. 9.—Fully developed motile form or vermiculus.

Fig. 10.—Fully developed motile form in process of disintegration.



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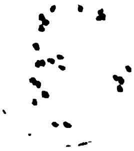

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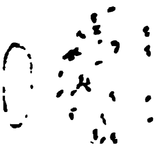

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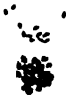

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PRELIMINARY OBSERVATIONS ON A CASE OF PHYSIOLOGICAL ALBUMINURIA.

BY TORALD SOLLMANN, M. D., AND E. C. McCOMB.
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PLATE XIII.

Introductory. Usually physiological albuminuria is of the kind designated by Pavy as "cyclic," *i. e.* albumin in notable quantity is at times present, at times absent. This form has been frequently studied and the conditions influencing it are fairly well known. Instances of this kind are undoubtedly physiological.

The present case belongs to the much rarer form, which stands on the very boundary of the physiological and pathological. It was characterized by the constant presence in the urine of a considerable quantity of coagulable proteids, for a considerable length of time, without any other symptoms of general or renal disease.

In February, 1896, one of us accidentally discovered the presence of albumin in his urine. He was at the time in perfect health. A physical examination, made by Prof. C. F. Hoover, failed to disclose anything abnormal. Nor did the ophthalmoscope reveal any trace of albuminuric retinitis. Chemical tests made at intervals since that time have always shown albumin. Frequent microscopical examinations have failed to reveal casts. The state of health at the present date (January, 1898) is still perfect, and there are absolutely no other signs pointing to the presence of renal disease. The weight is 154 lbs., height 5 feet 8 inches, age 27 years.

General Considerations. In view of the comparative uncertainty of our knowledge of the mechanism of urinary secretion, it seems to us that any contribution which could possibly throw some light upon the question would be valuable. The study of the conditions which influence the quantity of the normal urinary constituents has already yielded good results in this direction, but little has as yet been done in the way of making similar quantitative observations on the conditions influencing the excretion of proteids. The study of the present case therefore seemed to us of interest in this connection, as well as serving as a contribution to the knowledge of the different forms of albuminuria and the causes from which they result. In the present paper we content ourselves with giving the results of our work without attempting to apply them to the general questions suggested, as we are well aware that the number of observations is not sufficiently large nor are the results sufficiently unequivocal. The former defect we hope to remedy in the future. A table and a chart (Plate XIII) at the end of the article present the results of the observations.

The special questions which we attempted to answer are:

1. What is the nature of the proteids in the urine?
2. Is the albuminuria due to some general metabolic change or is it of renal origin?
3. What quantitative variations do the proteids undergo?

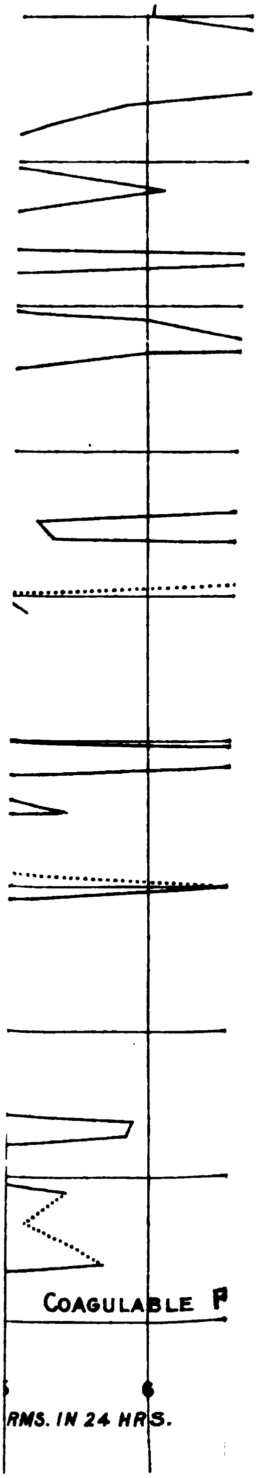
1. What is the Nature of the Proteids?

The proteid was found to consist of albumin and globulin. We at first attempted to determine the relative amount of these two substances, both by Pohl's and by Hammersten's method, but no satisfactory results were obtained, probably because the quantity of globulin was too small. We therefore contented ourselves with the estimation of the total coagulable proteid.

After the removal of the coagulable proteids the urine was frequently tested with sodium hydrate and cupric sulphate, but we could never obtain a biuret reaction, showing the absence of notable quantities of non-coagulable proteids.

2. Is the Albuminuria of Metabolic or of Renal Origin?

We attempted to answer this question by varying, as best we could, the conditions ordinarily accepted as influencing the general metabolism and the functional activity of the kidneys. Thus we examined the influence on the quantity of proteid excreted: *a*, of diet; *b*, of


COAGULABLE P
6
RMS. IN 24 HRS.

temperature; *c*, of sleep; *d*, of diuretics; *e*, of drugs acting upon the circulation; *f*, of spontaneous variations in the volume of the urine.

a. The effect of diet. The fact that the person under observation eats a good deal of meat and little carbohydrate served to direct our attention to this point.

It will be seen from the tables and curves annexed that, on the whole, the proteids in the urine vary directly as the proteids in the diet, *e. g.* urinary proteids from February 9 to 12 (with a diet poor in proteids) average .5384 grm. per diem; from February 13 to 14 (with a diet rich in proteids), .6430 grm. On closer examination it will be seen that the changes in the urinary proteids only appear the day after the change in the diet. Taking this into account, we have for diet poor in proteids (February 10 to 12) .4465 grm.; for diet rich in proteids (February 14) .6748 grm. The average of the preceding week (with normal diet) was .5083 grm. And on the whole the curves of urea and of coagulable proteids from February 9 to 19 will be found to correspond very closely.

But on the other hand, when we tried to keep the diet approximately uniform in quantity and quality (from February 25 to March 4), large variations were seen in the urinary proteids, which do not at all correspond to changes in the amount of urea.

b. The effect of temperature. The influence of external temperature can be well studied from the chart, in which the average and in some cases the minimum daily temperatures are given (from records of the U. S. Meteorological Station, Cleveland). It will be seen that, as a rule, the coagulable proteids in the urine vary inversely as the temperature, and this in a very striking manner (January 23 to February 6; March 11 to 23; April 14 to 22). Equally striking is the steady decline in the average daily excretion per month from January to April (see below under 3).

Occasionally, however, there are changes in temperature without corresponding changes in the quantity of excreted proteids (April 7 to 14). Or the changes may even be in the same direction in both (February 6 to 8, April 25 to 27); but here the periods were only of two days, and the results could easily have been influenced by other conditions which escaped observation. A cold sponge-bath of 10 minutes' duration (April 25 and 26) produced no rise in the amount of urinary proteids.

c. Influence of sleep. To determine the influence of sleep (rest and position?), we compared the average hourly excretion of proteids during the day and night, with the following results:

		Quantity of urine.	Quantity per hour.	Per cent. proteids.	Proteids per hour in grms.
April 6 to 7....	Day.....	570 cc.	38 cc.	.0520	.0198
	Night....	340	37.8	.0329	.01255
April 14 to 15..	Day.....	950	67.9	.0402	.0273
	Night....	370	37	.0557	.0206

It will be seen that during sleep the excretion of coagulable proteid is very notably diminished.

It would have been well to try the effect of severe exercise in this connection, but circumstances did not permit it at the time.

d. Influence of diuretics. In attempting to investigate the part which the kidneys played in the production of this albuminuria we tried (March 11 to April 9) the exhibition of various diuretics, viz. potassium nitrate, potassium acetate, caffeine, and urea, as a method of affecting the renal epithelium. On the whole these diuretics tended to cause a slight rise in the quantity of proteid excreted, but the effect was small and not always to be obtained. Even a dose of 31 grm. of potassium acetate produced very little change.

e. The influence of drugs acting upon the circulation. We chose trinitrin and digitalis for these experiments. The drugs were pushed until their effects were plainly felt. As will be seen from the curves, they tended to increase the excretion of proteid to some extent, but whether this action was due to changes in the renal circulation or to more general vascular effects, or to changes in the metabolism throughout the body or in some particular organ, the evidence at our command does not enable us to decide.

f. The relation between the quantity of urine and the quantity of proteids excreted. As we have seen, when the quantity of urine is increased by diuretics that act chiefly on the renal epithelium there is little change in the quantity of excreted proteid, but what there is is in the direction of a rise. The same is true of changes produced by diuretics acting chiefly on the circulation. But for those changes in the volume of the urine which, for want of a better name, we may call spontaneous, the case appears to be different, the total proteids sometimes increasing as the quantity of urine diminishes, and sometimes varying directly as the quantity. Thus, in looking at the curves from January 29 to February 6, a period during which no drugs were administered, one cannot fail to be struck with the fact that the maxima of the proteid curve correspond with the minima of the curve representing the changes in the quantity of the urine. From January 25 to January 29, on the other hand, the two curves run parallel. It may be of interest to mention in this connection that the observed individual was unable to drink large quantities of liquid.

3. What Quantitative Variations do the Proteids Undergo?

As will be seen from the curves, the variations in proteids during the three months covered by our observations were comparatively small, much smaller than we expected. The quantity in the twenty-four hours fell once to .2592, and once to .3242, and rose once to .9010, and three times it lay between .814 and .855, but did not stay at any of these extremes longer than one day.

Excluding these exceptional results, we found that the proteid varied from .7 to .37 grm. in 24 hours.

The average in January (10 days) was .6616; February (23 days), .5419; March (16 days), .5111; April (18 days), .4651.

The average for the 67 days observed was .5318 grm. On the supposition, which appears legitimate, that the average daily output for each month was approximately the same as the average of the days actually observed in that month, the daily average for the four months over which the observations extended would be .5449.

SUMMARY.

1. The proteids consisted of albumins and globulins, varying in quantity between the extremes of .9010 grm. and .2592 grm. in the

24 hours, but usually between .7 and .37 grm., the average being .5317 grm.

2. The quantity of the proteids varies directly as the urea, inversely as the external temperature. The relation between the quantity of urine and the quantity of proteid is not constant. The quantity of the proteids is little affected by diuretics; it tends to be increased by certain drugs that act upon the circulation. It is lessened during sleep.

It is apt to suffer a sudden temporary increase, returning as suddenly to the usual average. For this phenomenon we have no explanation.

3. From .5449 to .6616 grm. of coagulable proteids a day (.0079 to .0094 grm. per kilo of body-weight) may be excreted through the urine for an indefinite time by an otherwise healthy individual without damage to either kidneys or organism.

NOTE.

The method employed for the estimation of proteids was the usual gravimetric method with heat precipitation. It was found that the addition of acetic acid, even when done as carefully as possible, detracted from the exactness of the result, and with these urines it was generally quite unnecessary. The method as carried out consisted in heating a certain quantity (usually 100 cc.) of the urine on the sand-bath, bringing to the boil about five times, filtering through ashless filter paper, again boiling the filtrate, cautiously adding a few drops of 2 per cent. acetic acid, and, if this produced any turbidity (which it rarely did), refiltering and repeating the process until all the proteid was precipitated. The precipitate was washed on the filter with distilled water until chlorine-free, then with alcohol, and was dried at 110° C.

As the weather was very cold during the greater part of the time, we usually analyzed the urines of two successive days at the same time. In a few cases we added a small quantity of salicylic acid. Most of the analyses were made on the mixed urine of 24 hours, but some on the mixed urine of 48 hours. All the determinations were made in duplicate, and of course the higher of the two accepted. The difference between the two determinations averaged in 52 cases 3 milligrammes per 100 cc.; in four cases it was between 10 and 11 mg. per 100 cc.

TABLE OF RESULTS.

Date.	Quantity proteid.	Quantity urine.	Sp. gr. urine.*	Average daily temp.° C.	Urea.	Solids of urine calculated by Haeser's coefficient.	Remarks.
5 p. m. to 5 p. m.							
Jan. 19–20				(20)—8.3			
21				(21)—0.7			
22	.6045	930	27.5	—2.3		59.59	
23	.6045	930	27.5	—9.7		59.59	
24	.7357	880	28	—13.4		58.72	
25	.7357	880	28	—21.4		58.72	
26	.8397	1143	27	—18.7		69.21	
27	.8397	1143	27	—15.2		69.21	
28	.6000	750	29	—15.5		50.68	
29	.6000	750	29	—10.3		50.68	
30	.5279	1025	29	—8.4		69.26	
31	.5279	1025	29	—7		69.26	
Feb. 1	.4486	925	26	—2.8		56.05	
2	.4486	925	26	—2.3		56.05	
3	.6136	800	27	—2.8	23.276	50.72	
4	.6136	800	27	—2.3	23.276	50.72	
5	.4767	965	26	+0.3	29.734	59.05	
6	.4767	965	26	+1.9	29.734	59.05	
7	.4640	950	26	+0.4	28.781	57.56	
8	.4640	950	26	—1	28.781	57.56	
9	.8141	835	28	—1.7	27.791	54.47	Diet poor in proteids, rich in carbohydrates.
10	.4554	990	24	—1.8	20.766	55.36	Diet poor in proteids, rich in carbohydrates.
11	.4092	790	26	—3.3	20.648	47.86	Diet poor in proteids, rich in carbohydrates.
12	.4749	1060	23	—1.9	25.487	56.80	Diet normal in proteids, poor in carbohydrates.
13	.6111	970	24	—2.0	30.705	54.24	Rich in proteids, poor in carbohydrates.
14	.6748	1165	24	+1.9	34.281	65.14	Normal.
15	.7055	850	25	—0.2	30.517	43.49	Poor in both.

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* Only the last two figures of the specific gravity, as expressed in the ordinary way, are given.

TABLE OF RESULTS.—*Continued.*

Date.	Quantity proteid.	Quantity urine.	Sp. gr. urine.	Average daily temp.° C.	Urea.	Solids of urine calculated by Haeser's coefficient.	Remarks.
5 p. m. to 5 p. m.							
Feb. 16	.5197	1120	22	(16)+1	35.189	57.41	Much H_2O taken.
17	.4233	1135	26	+6.6	27.528	68.76	Little " "
18	.4368	1050	26	—0.3	28.355	63.61	
19	.4368	1050	26		28.355	63.61	
25	.7040	950	23.5	—3.6	21.780	52.01	Amount of food and water taken practically constant.
26	.5251	960	26	—7.8	24.027	58.16	
27	.5330	1300	22	—10.9	28.449	66.64	
28	.7360	1150	23	—7.5	26.838	61.63	
to March 1	.9010	1070	28	0	27.459	57.34	
2		1080	27	0.5	30.332	67.94	
3	.4963	960	27	3.3	28.541	60.39	
4	.5184	800	27	—1.1	28.472	50.72	
8 a. m.				(9) 12.2			
to Mch. 11	.3242	860	27	(10) 6.7		54.10	
12	.4828	1210	22	6.7		62.02	4 grm. pot. nitrate taken.
13	.4922	850	27	4.4		53.48	
14	.8554	720	30	—2.2		50.33	
15	.6296	660	31	2.8		48.33	4 grm. pot. acetate.
16	.4015	550	32	—3.3		41.01	
17	.4905	780	30	—3.3		54.52	
18	.5420	825	27	1.6		51.90	.6 grm. caffeine.
19	.3660	630	30	10		44.04	
20	.2592	810	30	10		56.62	
21	.3003	910	26	10		55.13	2.1 grm. urea taken.
22		1090	25	7.2		63.49	.4 grm. urea "
23	.6541	690	28	7.8		45.01	

TABLE OF RESULTS.—*Continued.*

Date.	Quantity proteid.	Quantity urine.	Sp. gr. urine.	Average daily temp.° C.	Urea.	Solids of urine calculated by Haeser's coefficient.	Remarks.
7 a. m.							
to Mch. 24	.4633	820	28	(23) 2.2		53.50	
25		980	27	2.2		61.65	11.7 grm. pot. acet.
				(5) 11.1			
to April 7	.4093	910	27	4.4		57.25	
8	.4408	1520	22	3.3		77.91	31.1 grm. pot. acetate.
9	.4392	900	24	3.3		50.33	
10	.3999	930	23	1.1		49.84	
11	.3927	730	28	3.3		47.63	20 min. tr. digitalis.
12	.4407	920	28	1.1		60.02	
13	.3993	920	26	3.3		55.74	
14	.3696	1200	21	12.2		58.79	.0039 grm. trinitrin.
15	.5880	1320	23	6.1		73.82	" " "
16	.5852	950	26	7.2		57.55	.0033 " "
17	.4804	1570	20	6.1		73.16	" " "
18	.4970	1000	23.5	1.6		54.75	
19	.4520	800	25	8.9		46.60	
20	.5440	1000	25	6.1		58.25	.0065 grm. strychnin [sulph.
21		1010	24	—1.1		56.48	.0065 " " "
22	.5131	1010	25	6.7		58.83	
25	.5694	1010	26	21.1		61.19	
26	.4567	1015	24	15.6		56.78	Cold sponge-bath, about 10 min., morn. of 25th.
27	.3949	1010	25	10.		58.83	

DESCRIPTION OF PLATE XIII.

See the text.

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EXPERIMENTAL MEDICINE

EXPERIMENTAL THYROIDISM.*

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PLATES XIV–XVIII.

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* This article was received by the editor in August, 1896, but on account of its length and the pressure of other material the publication has been delayed until the present time. In consequence of the active interest and research relating to the thyroid gland during recent years, this delay is much to be regretted. The date of completion of the article should be borne in mind by the reader. (Editor.)

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I. INTRODUCTION.

With the widespread employment in myxœdema of the thyroid gland and its various preparations that followed the reports of Murray, Horwitz, Mackenzie, and Fox, concerning the beneficial effects of thyroid extract in that disease, numerous other observers have also called attention in the past three years to the fact that in the course of such treatment certain symptoms are liable to arise. The symptoms consist of headache, dizziness, tachycardia, fever, nausea, vomiting, severe diarrhœa, fibrillary tremor and agitation, insomnia, urticaria, incomplete paraplegia, loss of consciousness, and finally general collapse. They are more or less liable to follow either the subcutaneous injection of the extracts, or the ingestion of large doses either of the fluid extracts or of certain commercial desiccated thyroid preparations.

In October, 1893, my attention was personally drawn to this subject by the fact that an adult man (Part I, C, Obs. 1, see below), who had previously taken large doses of unquestionably fresh, raw thyroid gland with absolutely no disagreeable after-effect, promptly developed evident symptoms of thyroid intoxication or "thyreoidismus," after taking daily for three days six five-grain thyroid tablets of Burroughs, Wellcome & Co. The difference in the action of the dried product when contrasted with the inactivity of the fresh gland was so striking in this instance that I determined to investigate fully the cause of this difference and, if possible, discover the source of the intoxication.

From my first observation referred to above I was led to believe that the toxic symptoms were probably caused by the action of certain toxic substances which had developed in the thyroid glands after their removal from the sheep. This single observation did not sufficiently demonstrate the correctness of the above opinion, and, in order to determine this and a number of points closely related to this subject, I began at that date the extensive series of experiments that are described in this monograph.

In a previous paper * dealing with one of the many corollaries of this subject I have already referred to the negative results obtained from feeding animals upon large amounts of fresh thyroid glands, but at that date, owing to the incompleteness of some of the comparative intravascular injection experiments and other experiments upon monkeys that are described also in this paper, I refrained from describing the special results that are given here. Previously to that date I had not only expressed the essential points demonstrated by my results to my friend Dr. S. J. Meltzer, but had in conjunction with him performed one experiment that resulted fully in accordance with my previous views upon this subject.

It must be noted that during the progress of this work Prof. Kocher † has intimated that possibly the thyroid intoxication may be due to the decomposition of the thyroid material; and later Lanz,‡ working in Prof. Kocher's clinic, has published his experimental investigations on this subject. This writer experimenting on himself and two friends noticed only an unimportant increase in the pulse rate to follow the ingestion of 20 to 30 grammes of fresh, raw thyroid. Having contrasted the effects of dried thyroid preparations by Merck, Haaf, and Burroughs, Wellcome and Co., upon mice, rabbits, dogs, and men, Lanz concludes that thyroidism consists of two components: "(*a*) einer Giftwirkung infolge Verarbeitung zersetzten Drüsenmater-

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* *Medical Record*, New York, June 15, 1895.

† Kocher, *Corr. Bl. f. schweizer Aerzte*, 1895, No. 1.

‡ Lanz, *Deutsche med. Wochenschr.*, 1895, No. 37.

ials, (*b*) einer für die Schilddrüse specifischen Wirkung. Letzterer, d. h. der Thyreoidismus im eigentlichen Sinne des Wortes, oder besser gesagt die Hyperthyreosis, äussert sich je nach der Provenienz des Drüsenmaterials und je nach der Art des Versuchsthieres in verschiedener Intensität ist quantitativ, aber nicht qualitativ verschieden."

I do not consider, however, that the experiments which this observer relates in his article indisputably warrant the latter of these conclusions, for a number of objections may be offered to them. Thus he found that mice die after the ingestion of either the decomposed dried thyroid material or the fresh material, requiring, however, very much larger doses of the latter. In these experiments the so-called fresh material appears to have been in reality a dried preparation that had been prepared as quickly as possible from the recently removed glands by drying *in vacuo,* pulverizing, and administering in the form of what Lanz terms "Totaldrüsenpillen." It is questionable, therefore, whether Lanz really employed the unaltered fresh material, for, before such material can be dried by this means, certain chemical alterations of the various proteid and other substances may readily occur. In fact I shall emphasize later that some of these substances do alter considerably in drying, and that even during a lengthy process of digestion, if large lumps of the unquestionably fresh raw gland have been ingested, a certain quantity of toxic material may possibly form in the central portions of the undigested pieces of thyroid. So exceedingly prone to early decomposition is the thyroid or "neck gland," that even the average slaughter-house butcher is generally acquainted with the fact.

The further consideration of other points dwelt upon by this author is deferred to another section.

On comparing my totally independent results with those of Lanz, I find that some of our experiences accord fully; others, however, do not, and in this paper I have endeavored, where possible, to explain the cause not only of these differences, but also of certain apparent clinical discrepancies that tend more or less still to surround this subject with some degree of uncertainty.

The original plan, therefore, upon which the present paper is framed is, first, to give a brief historical retrospect of certain previous important observations of others that bear more directly upon the numerous varieties of experiments performed by myself; second, to describe the objects, plan, and mode of research; third, to describe the experiments in definite series; and, finally, after comparing my results with those of others, to state the conclusions. The object being manifold, and the material rather extensive, I have determined for the purpose of description to divide the subject-matter of my experiments into parts, discussing in Part I thyroidism in healthy and diseased animals; in Part II the inquiry is made whether the appearance of the cachexia thyroidectomica is hastened or otherwise influenced by the ingestion of altered animal food stuffs; and in Part III are related certain comparative subcutaneous and intravascular injection experiments in which were used various organic substances or mixtures of substances that had been isolated from the thyroid and the thymus bodies.

II. RETROSPECT OF PREVIOUS OBSERVATIONS.

The first to use the thyroid juice for the purpose of investigating its physiological effect when injected subcutaneously or intravenously into animals seems to have been J. R. Ewald.* This observer removed the thyroid glands from a healthy dog, minced them, mixed them with 3 ccm. of warm water, and after the expiration of a few minutes pressed out the juice, which was filtered in some instances before using. This juice was then injected into a vein of another dog. Three hours after, this animal fell into a peculiar cataleptic condition which lasted one hour and a half. While in this condition the temperature was not increased or scarcely perceptibly so. In other experiments the subcutaneous method was employed with similar effects in some cases, but in others abscesses and non-absorption of the juice occurred. Ewald also states that similar, though less intense, effects were produced by extracts of the thyroid glands of other varieties of animals.

Previously to the above experiences of Ewald, Pellacani,† at first alone and later in conjunction with Foà,‡ had injected extracts from the liver, the suprarenal capsule, and the kidney of the calf into dogs, rabbits, guinea-pigs and frogs. After the subcutaneous injection of the extracts, agitation, vomiting, dyspnœa, somnolence, and general prostration appeared. The next morning the animal was paralyzed and death ensued later. When the extract was injected into a vein, dyspnœa, agitation, rapid heart action at first, which subsequently diminished in frequency, followed. In four hours the temperature had risen, and four hours later it fell, the animal dying shortly after. These writers concluded that these effects were common to many gland extracts. Mattei * and also Alexander † have disputed these observations. Langendorff,‡ however, repeated Ewald's experiments and found that the subcutaneous injection of thyroid juice produced effects similar to those observed by Ewald. Intravascular injection of the fresh juice produced rapid death. Intravascular clotting of the blood occurred in some of the animals, and in others the coagulability of the blood was lessened. Langendorff, in discussing his results, very appropriately refers to the practically similar effects that Pellacani, Foà, and Wooldridge § had noted as occurring with the extracts of other animal glands.

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* J. R. Ewald, *Berlin. klin. Wochenschr.*, 1887, March 14.

† Pellacani, *Arch. per le sc. med.*, iii, 1874.

‡ Foà and Pellacani, ibid. vii, 1884.

Alonzo,|| Gley,¶ and Horsley,** repeated Ewald's experiments, but with negative results. Horsley emphasizes the complication of these injection experiments by the presence of the "tissue fibrinogens" of Wooldridge, which are excessively toxic. Halliburton †† and Brodie ‡‡ studied the effects of injections of these nucleo-proteid substances and fully confirm the previous statements as to their toxicity.

In this connection the observations of Wagner, quoted by von Eiselsberg,§§ are interesting. Wagner injected mucin from the salivary glands of the ox into cats and produced agitation, tremors, and tetanoid spasms that more or less resembled those of the cachexia thyroidectomica. The great similarity of these symptoms to those produced by Foà and Pellacani by extracts of liver, suprarenal capsule, etc., is striking and suggests the possibility that a great number of animal substances exist that will produce similar symptoms when injected subcutaneously.

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* Mattei, *Arch. per le sc. med.*, vi, 1883.

† Alexander, Ziegler's *Beiträge*, xi, 1892.

‡ Langendorff, *Berlin. klin. Wochensch.*, 1889, p. 786.

§ Wooldridge, Ludwig's *Festschrift*, Leipzig, 1887.

|| Alonzo and Ughetti, *Riforma medica*, Oct., 1890, p. 1364.

¶ Gley, *Comptes rend. d. la soc. d. biol.*, 1891, p. 250.

** Horsley, Virchow's *Festschrift*, 1891, and *Brit. Med. Journ.*, Jan. 30 and Feb. 6, 1892.

†† Halliburton, *Journ. of Physiol.*, xviii, 1895.

‡‡ Halliburton and Brodie, ibid. xvii, 1894-95.

§§ von Eiselsberg, Sammlung med. Schriften von d. *Wien. klin. Wochenschr.*, No. 4, 1890.

On the other hand, Vassale * states that, after the intravenous injection of thyroid juice into thyroidectomized dogs, in three animals the symptoms of the cachexia diminished in intensity and the animals were saved for some length of time. Vassale concludes that the juice thus added simply sustains the animal until some accessory gland vicariously assumes the function of the thyroid. Similarly Gley † and Lanz ‡ found that some animals could be thus saved for some time, and the latter of these writers states that thyroidectomized dogs can be kept alive for months with 5 to 10 grm. of thyroid given daily by mouth. When this dose is stopped the tetanic attacks recur; and he also has noticed that the severity of these recurrent attacks diminishes more and more with the lapse of time after the thyroidectomy.

Historically bearing upon the validity of the conclusions that appear to be taught by the experiments of Gley and of Lanz are the well-known observations of Piana, Ewald, Wölfler, Wagner, Carle, Fuhr, Autokratow, Gley and others as to the frequent occurrence of accessory thyroid glands in the aortic and other regions. Gley, and Edmunds § also state that if the parathyroids (Sandström) are left in thyroidectomized dogs the animals live. I have seen young dogs live for several weeks after the removal of both lobes of the thyroid and the parathyroids that were partially embedded in them. In all of these animals either aortic or other accessory glands were found; in some were found several small accessory aortic glands which often were so minute that even one accustomed to the appearance of these bodies might easily overlook them.

In 1894 Ballet and Enriquez,|| endeavoring to produce experimental exophthalmic goitre in two dogs by the subcutaneous injection of the unboiled thyroid extract, claimed that besides fever, tremor and agitation, tachycardia and a swelling of the thyroid gland occurred. This goitre disappeared when the injections were discontinued, and reappeared when they were begun again. The extract used by these observers was a glycerine one, macerated for twenty-four hours and filtered through paper. They state that no abscesses followed the subcutaneous injection of this fluid in more than one hundred injections. The swollen thyroid exhibited ecchymoses on the surface and weighed 3.75 grm. The intestinal hæmorrhages they attributed to vasomotor origin from the fact that no

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* Vassale, *Centralbl. f. d. med. Wissensch.*, 1891, p. 14.

† Gley, loc. cit.

‡ Lanz, loc. cit.

§ Edmunds, *Proc. of Physiol. Society*, 1895, No. 4.

|| Ballet and Enriquez, *Sem. méd.*, xiv, p. 536, 1894, and Soc. méd. d. hôp. d. Par., 1894, pp. 805 and 941.

ulceration of the intestinal mucous membrane could be discovered. The state of the liver and the condition of the blood in the portal vein are not referred to in their paper. From similar experiments on dogs and rabbits Lanz * attributes these results to septicæmia following the injection of a decomposed preparation, and does not consider them as the symptoms of thyroidism. One of his animals developed an abscess in the parotid gland, and he infers from this that possibly a similar occurrence had misled Ballet and Enriquez. Furthermore the thyroid glands in Lanz's animals are stated to have become decreased in size to a considerable extent. In the hands of Edmunds † no visible effect was produced by the subcutaneous injection of the thyroid extract (mode of preparation not described) into normal monkeys. Of thyroidectomized monkeys, out of eight treated with injections none recovered.

Up to the present I have confined my remarks to the negative and positive results derived from experiments upon the lower animals, but after Murray ‡ in 1891 recommended the subcutaneous injection of a macerated glycerine extract of sheep's thyroids for the treatment of myxœdema, many clinical announcements appeared calling attention to certain dangerous symptoms that were liable to occur during the treatment. Thus Murray § and others agree that the injections were followed in some instances by nausea, flushing, pain in the lumbar region, loss of consciousness, and symptoms of collapse. Mackenzie || and also Fox ¶ gave the extract or the raw gland by the mouth in order to avoid the dangers consequent to the injections. Mackenzie states in his summary that the acceleration of the pulse and the rise of temperature following the administration of thyroid are proportional to the amount ingested. Fox gave both the glycerine extract and the fried minced gland, and produced in a myxœdematous patient at the expiration of a fortnight profuse perspiration and inability to walk or stand steady.

Kocher and other observers did not observe such toxic symptoms to follow even large doses of the fresh raw gland. Bruns ** gave doses of 5 and 10 grm. of fresh thyroid from lambs or calves. In one case only did headache, nausea, and loss of appetite occur. This patient took 46 grm. in 14 days, during which period a loss of 10 kilos in weight occurred.

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* Lanz, loc. cit.

† Edmunds, loc. cit.

‡ Murray, *Brit. Med. Jour.*, 1891, p. 796.

§ Murray, ibid., 1892, p. 449.

|| Mackenzie, *Brit. Med. Jour.*, 1892, p. 940.

¶ Fox, ibid., 1892, p. 941.

** Bruns, *Deutsch. med. Wochensch.*, 1894, p. 785.

In the other cases reported by Bruns no unpleasant disturbances resulted, although the amount of raw thyroid taken was greater. Kocher * gave 10 grm., and often larger doses, of fresh thyroid without evil effects and unaccompanied by any diminution in the bodily weight; in fact the weight often increased. The only effect of the fresh raw thyroid in doses of 20 to 30 grm. that Lanz noticed in himself and in two friends was an unimportant increase in the pulse rate. The length of time that such doses were continued is not stated nor are the other details of these experiments described in Lanz's paper.

Sonnenburg's † experience with the raw gland in a case of cachexia thyroidectomica in which a small portion of the gland had been left is also very instructive. His patient took 15 grm. of fresh minced calf's thyroid on February 6, 7, 8, 9, 13, 14, 15, 16, 23 and 28, also on March 2 and 8. During the month of March she took fourteen 15-gramme doses of thyroid. The temperature became normal in February and remained so. The weight at the beginning of February was 37 kilos; on the 19th, 37.75 kilos; and on March 3rd the weight had reached 40.2 kilos. Sonnenburg's results differ markedly from the opinions promulgated by numerous later writers regarding the special susceptibility of myxœdematous cases to the early occurrence of the symptoms of intoxication during the exhibition of various thyroid preparations.

Béclère ‡ reports the following marked instance of acute thyroidism. 92 grm. of sheep's thyroids having been taken in 11 days by a myxœdematous patient, the myxœdema symptoms were replaced by those of thyroid intoxication, which consisted of agitation, tachycardia, insomnia, increased temperature and respiration, exophthalmos and incomplete paraplegia. In short a rather severe degree of the so-called thyroidism was produced. No particulars, however, are given either as to the source of the glands or as to their absolute freshness. Nor do the majority of clinical reporters of thyroidism from the raw gland particularize as to the absolute undoubted freshness of the material which had been given. In a number of instances the patient has procured the glands from the family butcher, who often removes them from carcasses that have been hanging in his shop or in his ice-box for one day or more. Such thyroid glands are certainly not fresh in the strict sense of the term. Probably putrefaction has not occurred, but the rapid and well known post-mortem alteration has occurred. In warm weather the dead glands will give an acid reaction even within three hours after death, and

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* Kocher, loc. cit.

† Sonnenburg, *Arch. f. klin. Chir.*, xlviii, 4, p. 857.

‡ Béclère, *Mercredi méd.*, 1894, p. 511.

often a perceptible odor is noticeable after six hours in spite of the fact that the glands have been kept in an ice-chest. In fact one of the most vexatious obstacles that was met with in conducting these experiments with the raw gland was the difficulty of procuring large amounts of the perfectly fresh glands and utilizing them within a few moments after their removal.

After the advent of the various commercial desiccated prepartions of sheep's thyroids put upon the market by a great number of firms, the occurrence of mild or of severe thyroidism is more frequently noted in the clinical reports. Bramwell, Abraham, Kocher, Leichtenstern, Meltzer, Ewald, Lanz, and a host of English, French, German, and American observers, all relate experiences more or less similar. Briefly summed up, one learns from their reports that the activity, toxic and alleviative, of such commercial desiccated preparations is exceedingly variable, but all of these writers unite in urging the employment of great care at the beginning of the administration of such preparations, in order to avoid, if possible, the effects of overdosing.

Lanz, apparently considering a certain dried preparation as equivalent to the fresh gland, relates several comparative cases as illustrative of his views regarding the causation of at least the greater part of the symptoms of thyroidism by the decomposition products which the various other dried commercial preparations are concluded to contain. For instance, to a case of goitre was given on March 2, two "Totaldrüsenpillen" (Haaf), each equal to .2 grm., and the dose was increased by one pill each day until March 8. From March 9 to March 13 no thyroid was given, with the result that the pulse fell from 104 to 84. On March 13 ten more pills were given, and repeated thereafter with a daily additional increase of two pills. On March 16 the pulse was 104-108 and temperature 38.1° C. No other symptoms occurred. In another case when the progressive dose had reached 10 grm., and this amount had been given twice, the patient complained of headache, lassitude and sleepiness. In other cases as much as 9 grm. of this preparation per day were required to produce the first symptoms of disturbance. With the preparation of Burroughs, Wellcome & Co. even 1 grm. per day could not be borne longer than one week without causing headache, excitement, loss of flesh, etc.

In his experiments on mice Lanz also employed the "Totaldrüsenpillen" preparation, ultimately with fatal effect, finding that the English preparation in doses of even less than 1 grm. caused death, whereas the mice in some instances required total amounts of 50 grm. of the dried fresh calf thyroid to produce the same effect. These results demonstrated nothing in my opinion but the fact that the toxicity of the various dried thyroid preparations varies greatly in degree.

Even the latest evidence, therefore, does not satisfactorily answer the question as to whether the absolutely fresh raw thyroid material is toxic or harmless. I submit, therefore, in the following pages the results of my own independent investigations on this subject.

III. OBJECTS AND PLAN OF RESEARCH.

Bearing in mind the confused evidence that existed in the literature up to October, 1893, as to the toxicity of the thyroid material, and comparing the experiences of previous clinical workers with the results of my first personal observation with the fresh raw gland (page 178), I aimed primarily:

(1) to determine conclusively whether or no the fresh raw thyroid material is toxic either to man or to the lower animals;

(2) to investigate the relationship of the symptoms of induced thyroidism to those of Graves' disease;

(3) to observe whether or no the toxic thyroid or other animal material influences the appearance of the cachexia in thyroidless animals;

(4) to study the effects of various substances extracted from the thyroid, thymus and other glands upon thyroidless animals, with the possible result of ultimately isolating the active substance that the thyroid body is considered to contain.

Briefly summarized, I proposed to elucidate the above-mentioned objects:

(*a*) by producing in various animals acute or chronic thyroidism and noting in every instance the character of the thyroid material employed;

(*b*) by also endeavoring to produce in animals a train of symptoms similar to those of thyroidism, employing for the purpose animal material other than the thyroid gland;

(*c*) by producing the cachexia thyroidectomica and noting the susceptibility of these animals to the thyroid and other animal material as well as to special substances that had been chemically isolated from this material;

(*d*) by administering the thyroid material in a certain disease that is claimed by many to be due to over-activity of the thyroid gland.

Lastly, it has been deemed advisable to repeat with some modifications certain intravascular injection experiments of Schäfer * and Oliver.† My reasons for so doing are given in full in Part III.

Naturally during the progress of my numerous experiments many more or less important and interesting questions in connection with the previous thyroid work of others came to light, but their enumeration is deferred until I have discussed the points upon which they bear directly.

IV. OPERATIVE PROCEDURE AND METHODS.

In every instance in which thyroidectomy was performed the animals were anæsthetized with ether and the strictest aseptic and antiseptic precautions observed throughout the operation. In all the intravascular injection experiments ether was also employed. In these latter experiments I frequently administered a large preliminary dose of morphine also, and at the end of each experiment the still anæsthetized animal was painlessly killed by an overdose of the anæsthetic.

In order to produce the intoxications I administered the thyroid or other animal material either by subcutaneous injection or by mouth. My mode of procedure by the latter method was as follows:

Birds. Small pieces of thyroid, meat, capsules, tablets, etc., were placed on the back of the tongue and the bird was allowed to swallow them. Liquid extracts, bouillon and powdered thyroid suspended in water were injected into the œsophagus through a rubber tube attached to a syringe of large capacity.

Rabbits. Some of these animals would chew the tablets and swallow them. Usually it was necessary to powder them, suspend in water, and give by the tube. The raw material, after it had been minced very fine with a mechanical meat grinder, was mixed with a small quantity of distilled water and injected into the stomach with the tube and syringe.

Carnivora. Cats and dogs would readily eat large amounts of the fresh material; the dogs often considerable quantities of even putrid thyroid. Sugar-coated tablets and capsules that had been dipped in syrup were taken with avidity by the dogs; one of the dogs consuming 300 tablets in a few minutes and whining for more. Large quantities of powdered material, liquid extracts and defibrinated blood were administered through the stomach tube.

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* Schäfer, Internal Secretion. *Brit. Med. Jour.*, Aug. 10, 1895.

† Schäfer and Oliver, *Jour. of Physiol.*, xvi, 1894; xvii, 1895; xviii, 1895.

Monkeys. In all instances the tube was necessary and only inserted after previously cocainizing the fauces with a momentary spray of 1 or 2 per cent. solution of cocaine. Owing to the ease with which these animals vomit they were kept under close observation for a number of hours after the dose, and if vomiting occurred, the fact was specially noted and the dose not counted.

Man. The perfectly fresh raw thyroid material was given always in a very finely minced state, after wrapping in gelatine wafer papers. The dried commercial preparations were taken in the form of capsules or tablets.

In order to produce the intoxications the following varieties of material were used:

(*a*) Raw thyroid, fresh, stale, or putrid, from the sheep, pig, calf, ox, and in a few instances from the dog.

(*b*) Raw thymus and suprarenal capsules, fresh, stale, or putrid, from the calf and ox.

(*c*) Fresh thyroid, rapidly dried *in vacuo*, from the sheep.

(*d*) Dried commercial thyroid from the sheep; a great number of different batches from five different firms being tested.

(*e*) Meat (muscle, etc.), fresh, stale, or putrid, from the ox.

(*f*) Aqueous extracts, glycerine extracts, and bouillon from *a*, *b*, *c*, *d*, and *e*.

(*g*) Special substances or mixtures of substances chemically isolated from *a*, *b*, *c*, *d*, *e* and *f*.

(*h*) Defibrinated blood of thyroidless dogs removed during the acme of the cachexia.

(*i*) Sundry commercial so-called nuclein preparations and bouillon prepared from them.

Preparation of extracts.—At the outset of the investigation Murray's method was followed;* later I omitted the carbolic acid and the glycerine, and extracted with water only or with a normal saline solution, in some instances adding a few crystals of thymol as a mild preservative. These extracts were exceedingly easy to prepare, but rather too crude for aseptic subcutaneous injections. The aseptic fresh extracts were therefore prepared as follows:

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* Murray (*Brit. Med. Jour.*, Oct., 1891) prepared the thyroid extract by grinding the sliced glands with bits of ordinary glass tubing in a mortar. After thorough disintegration a fluid drachm of a mixture of equal parts of sterilized glycerine and a five per cent. aqueous solution of carbolic acid was then added, and when well triturated the mixture was macerated for twenty-four hours. At the expiration of this period it was filtered through a double layer of sterilized paper under pressure, and of the nearly clear, pale yellow filtrate 15 minims were employed for each injection.

A known weight of finely ground material, absolutely fresh—the animal having been slaughtered 10 or 15 minutes previously—was rubbed in a mortar for 15 minutes with an equal weight of boiled distilled water. This mass was placed in a stout, fine-meshed cloth and the juice expressed by a powerful press. This juice was diluted with an equal volume of water to facilitate filtration, quickly filtered through coarse paper by means of a vacuum pump, and finally filtered through a Chamberland candle. By using large quantities of material and employing a number of candles I could procure daily sufficient fresh fluid for my purposes. 2 ccm. of this solution was assumed to be approximately equivalent to 1 grm. of the raw material. Throughout the investigation it has been my aim for the sake of simplicity to maintain the strengths of my extracts that were *not filtered* through the Chamberland candles so that 1 ccm. of liquid would equal 1 grm. of the raw material. I have thus invariably injected or given twice the noted cubic centimetres of the extracts that had been filtered through the candles. When it is stated that the animal received 15 ccm. of the filtered (Chamberland) extract (1 ccm. = .5 grm. of gland), I mean in reality 30 ccm., or the approximate equivalent of 15 grm. of the raw material.

In preparing the bouillons the thyroid, thymus and meat were also ground as fine as possible, covered with an equal weight of water, and boiled for different periods of time, water being added from time to time to supply the loss from evaporation. After cooling and skimming off the grease the fluid was filtered usually through paper, and the filtrate was then ready for use.

In many instances I found the administration of these bouillons much facilitated by previously evaporating them to one-half or one-fourth of their original volume.

Throughout this article I have applied the term " fresh " to the material only when I was positively sure that such was the case. Our laboratory attendant having witnessed the slaughtering of the animals, the thyroid or other material was immediately removed and forthwith conveyed to the laboratory, where it was prepared for use as rapidly as possible. Generally speaking, within twenty to thirty minutes from the time of their removal I had ground the glands and given them to the test animals.

In certain experiments in which the effects of the fresh material were compared with those produced by the same material after it had been kept for different periods of time, I proceeded as follows:

Each piece of fresh substance was halved and each half placed in a separate vessel. After weighing, one portion was ground and immediately administered. The remaining unground portion was put in the ice-box for twelve or more hours, then ground, and finally given to an animal as nearly like the preceding one as possible. This method of control consequently enabled me to give the animals approximately the same material in different states of freshness.

Regarding the mode of preparation of the various commercial dried preparations * that I have employed, naturally I am in the dark. Judging merely from the appearances and odor of the products one would conclude that the different firms employ very different methods. Nearly all of them appear, however, to contain large quantities of common salt, a circumstance that caused considerable trouble in a number of instances with the monkeys.

The description of the preparation of the special substances from the thyroid, thymus and other material is postponed to Part III.

V. EXPERIMENTS.

In the following description of my experiments I do not give in detail every experiment performed, for this would make this article unnecessarily voluminous and filled with wearisome repetition. I wish it distinctly understood that the results of the experiments upon which my conclusions are based have been repeatedly obtained, and when I have made an unrepeated observation the fact is distinctly stated. Many of the animals were utilized for a number of different experiments, but a week or more was usually allowed to elapse from the end of one experiment to the beginning of the next, the animal being carefully observed in the interim. Sufficient time was thus allowed for the complete elimination of the preceding preparation.

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* In this connection I take occasion heartily to thank Messrs. Fairchild Bros. & Foster for the large amounts of their thyroid preparations so kindly furnished. Also I am deeply indebted to Mr. Von Holland, of the Eastman's Supply Company of New York, for kindly supplying the greater part of the fresh material that has been used in the course of my experiments.

PART I. THE PRODUCTION OF THYROIDISM IN NORMAL AND IN DISEASED ANIMALS.

A. *The subcutaneous injection of thyroid and other extracts in normal animals.*

In spite of the previous observations of Foà, Pellacani, Wooldridge, Horsley, Langendorff and others relative to the complications concerned in such experiments, I wished to satisfy myself fully as to whether the absolutely freshly made crude extracts would produce the ultimate lethal effects that these authors ascribe to the various extracts in general. A few tentative experiments were thus performed on rabbits and dogs, but owing to the occasional occurrence of abscesses at the points of injection, I abandoned the crude extracts for the aseptic varieties, viz. those that had been filtered through a Chamberland candle.

Though abscesses very rarely followed the injection of these aseptic, unboiled, aqueous extracts, the ultimate effect of large doses of the various extracts in general was uniformly fatal, some of the rabbits dying in less than one hour from 30 ccm. (= 30 grm. of material), others not until the following day or the day after. Decreasing the doses I found that some of the rabbits died in about six hours from one injection of 10 ccm. of thyroid extract, others withstood this amount for several days and died usually during the night. With doses of 3 to 6 ccm. death did not so frequently occur. Some of the animals withstood these small daily amounts for 6 or 10 days, finally dying during the night, or after manifesting the usual symptoms on the day preceding death.

If the injections were discontinued after the onset of mild toxic symptoms several of the rabbits recovered completely, even though considerable paralytic weakness had developed in the legs.

When the aseptic thymus extract was used my average results were about the same.

With extracts from material macerated from 12 to 96 hours the rabbits succumbed to much smaller doses, the objective symptoms being possibly more pronounced. Similar extracts prepared from the sugar-coated thyroid tablets of Burroughs, Wellcome & Co., the only commercial preparation procurable at the time the above experiments were made, appeared remarkably active, 3 grm. often proving fatal to a rabbit in one or two hours.

When very large doses of the fresh aqueous extracts were injected, within 20 minutes in two instances, the animals exhibited fibrillary muscular twitchings, rapid respiration, accelerated pulse, inability to sit up, the animal lying sprawled out on its belly, and marked exophthalmos in many instances. If the dose was small some of these animals recovered; if the dose was large the animal became apparently paraplegic, the paralysis seeming to be due more to muscular weakness than to a central disturbance. Finally, a general paralysis, one or two slight fits, coma, and death supervened. The rectal temperature of some of the animals was investigated and found in the early stages either normal or very slightly increased. If an extract from stale or dried thyroid or thymus had been injected the temperature was usually markedly increased. Frequently in the stage of coma the temperature fell 1° F., or even 1.5° F. below the normal.

When the dose of the extracts was small and given daily, the animal in the course of a few days became much depressed and refused food. As time elapsed emaciation and great general weakness became prominent. The temperature rose to 105° F. or even higher. The hind legs became very weak and the animal appeared paralyzed, the knee jerks often remaining present, however. In two rabbits slight exophthalmos was observed. Finally, a slight, or severe, watery hæmorrhagic diarrhœa developed and the animals promptly died.

The results of my experiments with dogs with all of these filtered, unboiled extracts were practically the same, much larger doses naturally being required to produce a fatal result. Dogs as a rule withstood the injections for longer periods of time, and several of these animals, even after becoming paraplegic, fully recovered when the injections were stopped. One hardy animal withstood 25 ccm. of fresh filtered thyroid extract administered daily, except Sundays, for eighteen days, but finally succumbed with the usual symptoms.

The following experiment will indicate the average result:

Exp. I. Four yellow mongrel dogs of about the same weight were chosen. Dog 1 received daily injections of 20 ccm. of fresh thyroid extract and died on the sixth day, having exhibited during life considerable emaciation, great weakness; paralysis of all four extremities, more marked, however, in the hind legs; a temperature of 106° F.; pronounced thirst, and numerous bloody evacuations.

Dog 2 received 20 ccm. of a fresh filtered thymus extract daily for eight days and succumbed, manifesting symptoms identical with the above. On the day preceding the death of the animal a slight exophthalmos and considerable conjunctivitis were noticed.

Dog 3 was injected daily with 10 ccm. of a filtered thyroid extract prepared from raw material that had been kept twenty-four hours in an icebox. It died after the fifth injection.

Dog 4 got 10 ccm. (= 10 grammes of raw gland) of a filtered extract prepared from some sugar-coated tablets of Burroughs, Wellcome & Co. I note by the way that this particular batch of tablets had produced severe toxic symptoms in man. Result: died twelve hours after receiving the third injection. The symptoms manifested by this dog differed somewhat from those of the other animals. The emaciation was more marked. The fibrillary muscular twitchings were extremely prominent. The hind legs were stiff and kept rigidly extended. Voluntary movement appeared to be completely lost, but cutaneous sensation was present. Considerable cyanosis accompanied by very marked exophthalmos finally set in and after two slight fits the respiration ceased.

The lesions found both in rabbits and in dogs differed only in degree and were as follows:

The composition of the blood appeared to have been greatly altered. Usually very peculiar friable clots were found in the portal vein and its tributaries. Numbers of large or small intestinal ecchymoses were present either in the mucous membrane or beneath the intestinal peritoneum. The liver frequently contained hæmorrhages and the surface was often mottled with small hæmorrhagic areas. The kidneys were deeply engorged in some animals, but appeared normal in others. Sometimes the medulla of the suprarenal bodies was completely disintegrated and the seat of a hæmorrhage. Abscesses of the salivary glands, such as Lanz has noticed, did not occur in any of my animals. The thyroid lobes in three dogs were the seat of superficial or interstitial ecchymoses. These accumulations of blood would naturally increase the weight of these bodies, but a microscopical examination showed that no apparent hypertrophy was present such as Clark, Greenfield, Bamberger, Mackenzie and others state as occurring in exophthalmic goitre in man. In neither rabbits nor dogs did I get the marked diminution in the size of these bodies that Lanz observed in his animals, but the further detailed discussion of this latter circumstance is included in a later sub-section.

From the above I was convinced that subcutaneous injection of the unboiled, crude thyroid extracts is lethal, provided the dose be large, or small and frequently repeated. Having eliminated possible complicating septic and pyæmic effects in the latter instance by using aseptic extracts that had been quickly prepared from absolutely fresh material, it was concluded that ordinary septicæmia was not a necessary factor in the intoxication.

Comparative experiments with the aseptic extracts of other glands, and commercial nucleo-proteid preparations, have shown me that an intoxication accompanied by the same symptoms and lesions will likewise occur when these latter substances are injected. The symptoms of the so-called thyroidism can thus be produced by the subcutaneous injection of extracts of other glands.

Extracts from material that had been kept for twelve hours or more appeared more active. The extract from the above-mentioned thyroid tablets was similarly exceedingly toxic; but after keeping this and the other extracts until very marked putrefaction had occurred, and refiltering through the candle, the toxicity appeared to become considerably lessened.

Having, therefore, evolved by this means practically no more facts than previous observers have noted, the subcutaneous injection of these complex mixtures was abandoned and I concluded that I could not thus gain definite information either as to the specific toxicity of the unaltered thyroid material, or to the relationship of increased thyroid secretion, or of disturbed thyroid function (Moebius), to Graves' disease.

B. *Thyroidism from the ingestion of thyroid material.*

1. *Administration of fresh glands to normal animals.*

Many clinical observers having established the fact that thyroid juice from the sheep, pig, calf, or young ox, is effective in myxœdema, material from similar sources was employed in the following observations.

Generally speaking, I began the experiments on Mondays and gave daily doses for six consecutive days, skipping the seventh day on account of my inability to procure absolutely fresh glands on that day. During the following week, and often in the third week, the same, or larger amounts even, were continued in order that the test might be as fair as possible. This period of time was deemed sufficient for the effects of possible cumulative action to appear. The rectal temperature, the pulse, the respiration, the weight, the condition of the thyroid body, and the condition of the eyes, were carefully followed in numbers of animals from day to day. For the purpose of more accurately determining the effect of the thyroid feeding upon the normal thyroid gland, I quickly and carefully exposed and measured the thyroid lobes of several etherized dogs before the administration was begun. On exposing these bodies at the end of the experiment, preparatory to their removal, measurements were again taken *in situ* and the two measurements compared.

Owing to the fact that animal food-stuffs may undergo purefactive alteration in the intestines of rabbits and monkeys as well as in those of man, I invariably introduced the fresh material in an exceedingly finely divided state, hoping thereby to facilitate the digestion and absorption of the entire dose in the shortest possible time.

Obs. 1. A young cock; weight 2520 grm.; pulse 142; temperature 108⅖° F.; was given 50 grm. per day of fresh sheep's thyroid for sixteen days. The total amount received by this bird was 700 grm. At the end of the experiment the weight was 2530 grm.; otherwise no effect had followed.

Obs. 2. Two rabbits were given 40 grm. of fresh calf's thyroid daily, except Sunday, for twelve doses. Two more rabbits received 800 grm. each of fresh sheep's thyroid in 18 days, in doses of 50 grm. per day. These rabbits decreased from 30 to 75 grm. in weight, but this fact is not surprising, for in order that the stomach should be empty at the time of the administration the animals were allowed to eat only from midday to six o'clock in the afternoon. The effect was negative in other respects. Other similar experiments in rabbits yielded results almost identical with the above.

Obs. 3. Having fed for two or more weeks several dogs with daily doses of as much as 300 grm. of fresh thyroid, I gave to one of these animals nothing in the way of food for six days except a mixture composed of chopped thyroids of the ox, calf and sheep. In every instance the animals gained in weight, one dog increasing as much as 1 kilo. In no animal was the temperature disturbed or diarrhœa produced. The animals were all so lively and jumped about so much that often considerable difficulty was experienced in getting the pulse rate proper to the state of rest. Numbers of careful daily estimations demonstrated conclusively that no acceleration or irregularity had been produced.

Obs. 4. On the normal cat but one feeding experiment was performed. This experiment yielded a negative result after eighteen daily doses of 50 to 60 grm. of mixed sheep and calf thyroids had been given.

Monkeys. Obs. 1. Java monkey (Macacus cynomolgus) with moderate horizontal nystagmus; weight 1357 grm.; pulse rate 106; rectal temperature 101⅜° F. 90 grm. of fresh sheep thyroid were given to this animal in three days. At the end of this time the temperature was 101° F., pulse rate 100, and the weight 1354 grm. On the fourth and fifth days the animal received doses of 55 grm. of the fresh raw thyroid, but without disturbance of its general health.

To several other Java monkeys, one Bonnet, one Rhesus, and a Capuchin monkey, doses of 30 to 35 grm. of fresh sheep thyroids were given daily except Sunday for one to three weeks. In none of these latter monkeys did toxic symptoms arise.

Obs. 2. A large Java monkey; weight 2230 grams; temperature 102½° F.; pulse about 160 (animal much frightened); was given 15 grm. of fresh young ox thyroid. The next morning the temperature was 102, the pulse 150; the animal being still much frightened. It was then given 43 grm. more, and daily thereafter the following amounts: third day, 55 grm.; fourth day, 75 grm.; fifth day, 55 grm.; sixth day, 57 grm. (T. 101⅜° F.; pulse 110; weight 2265 grm.); eighth day, 65 grm. On this date the temperature was 101⅛° F.; the pulse 110; and the weight after taking the above dose 2280 grm. On the ninth day it received 67 grm.; tenth day, 75 grm.; eleventh day, 57 grm.; twelfth day, 63 grm.; thirteenth day, 67 grm. The animal now weighed 2271 grm.; the temperature was 101° and the pulse 96. The thyroid administration was then suspended and two days later the rectal temperature registered 102° F. The large doses ingested by this animal produced therefore not the least manifestation of the so-called thyroid intoxication.

From these experiments it would seem that the temperature of some of the monkeys to whom large doses of the fresh gland were given did appear to diminish somewhat and remain below the normal during the administration. Thus in the monkey quoted in observation 2 the temperature registered 102⅛° F. before any thyroid was given. During the experiment it seemed to fall progressively, and on the day that the experiment was discontinued had reached 101° F. Two days later it had become 102° F., and has apparently remained between this and 102⅛ for some weeks.

On the other hand I have noticed that the rectal temperature of many of the monkeys to whom nothing except their usual food had been given would often vary from day to day. The morning temperature of some of these monkeys would be as low as 99° F., while the afternoon temperature would register from 101⅜° F. to 102⅞° F. Again, in bringing the animals from their house to the laboratory where the daily doses were administered and the temperatures taken, an unavoidable though short exposure to the open air was necessary. I am unable consequently to determine definitely whether the diminution noted resulted from the thyroid material or was merely an ordinary variation from natural causes.

The practical absence of effect on the bodily weight is noteworthy and contrasts greatly with that produced in monkeys by much smaller though equivalent doses of the dried commercial thyroid.

Man. Naturally the experiments described below were conducted in every instance with due precaution, and daily personal observations were made, so that should the least symptoms of the intoxication arise the treatment could be discontinued immediately.

Obs. 1. Adult man, aet. 45 years; weight 205 pounds; pulse 80; axillary temperature 98⅜° F. Moderate obesity, but otherwise normal. Digestion excellent. Took daily for four days 8 grm. doses of fresh sheep's thyroid, the material being from a lamb that had been slaughtered but a few minutes. No effect was noticeable. From May 22 to June 4 daily amounts varying from 40 to 60 grm. were taken, the fresh thyroid being always finely minced and rolled in one or more gelatin wafer papers. Result: pulse 80; temperature 98⅞ F.; weight 207 lbs.

The fresh thyroid was then replaced with the commercial tablets and the effect of small doses of these is described below.

Obs. 2. An adult man, weighing 190 lbs., took daily except Sunday eighteen 16-grm. doses of fresh calf's thyroid. During and at the end of this treatment no perceptible alteration either of the pulse rate or the temperature could be determined. The man gained one pound in weight, however.

The question may arise as to whether these men really received the thyroid gland and not some other gland, such as thymus or salivary glands. Starr,* Owen † and others relate such occurrences, and in fact such a mistake is rather liable to occur if the choice of the material be left entirely to the discretion of the family butcher. The thymus, "neck sweetbread," or "throat bread," is well known to the average butcher, but the thyroid or "throat glands" are not so generally known, and are in fact often regarded by certain butchers as bits of muscle. I am fully satisfied that all the men for whom I have prescribed the fresh thyroid did get the correct thing, for the slaughter-house butchers who supplied the above men had removed numbers of thyroid glands for my personal use and were well acquainted with the two lobes. Moreover, at my request, the above men brought me from time to time samples of what they were taking.

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* Starr, M. A., *Med. News*, Dec. 15, 1894.

† Owen, David, *Brit. Med. Journ.*, Feb. 16, 1895.

From the above observations it appears that normal cocks, rabbits, cats, dogs, monkeys and men manifest no toxic symptoms whatsoever *after* the ingestion of large amounts of the finely ground absolutely *fresh* raw thyroid substance. Further, no exophthalmos, tremor, increased temperature or acceleration of the pulse could be detected, and the body weight was diminished, and that immaterially, in rabbits only.

2. *Thyroidism from stale or dried material.*

Rabbits. (*a*) *Administration of thyroid.* Obs. 1. Several sheep's thyroids were halved and one of the portions was put in the ice-box for twenty-four hours. Of the fresh portion 50 grm. were immediately given at 10.30 A. M. to a rabbit. Of the second or stale portion 50 grm. were also given to a second rabbit. The first rabbit manifested no ill effects, but the second died during the night, evidently having had a severe diarrhœa. The experiment was repeated, but 30 grm. only of the stale material were given to the second rabbit. In this instance the first rabbit showed no visible disturbance; the second, however, died in about twenty-four hours, exhibiting diarrhœa, marked weakness of all four extremities, dyspnœa and cyanosis, exophthalmos and coma, followed by death.

Obs. 2. A large rabbit was given 30 cc. of a water and glycerine extract from glands twenty-four hours old. The death of the animal followed in six hours, after it had manifested the usual symptoms except exophthalmos. A smaller rabbit that had received 50 grm. of the same material in the fresh state on the previous day showed nothing.

A third rabbit was given 20 ccm. of a glycerine extract prepared from glands that had been kept a similar length of time, with the result that on the following morning the animal was dull, took but little food, had an increase of 1.5° F. in temperature, and had lost 20 grm. in weight. After another 20 ccm. dose of the same extract it succumbed during the night.

Obs. 3. Exp. 1. Rabbit; weight 1680 grm.; given 1 grm. (11 grains = 1 sheep's thyroid?) of a dried commercial preparation. Two days later the animal weighed 1540 grm.; it was dull and refused food, and the temperature had risen 1° F. 3 grm. of the same preparation were administered in the afternoon and the animal died during the night, leaving signs of having had a very severe hæmorrhagic diarrhœa. The weight of the body after death was 1380 grm.

Exp. 2. Brown rabbit; weight 1705 grm.; temperature 101⅜° F.; was given 6 grm. (6 grains = 1 average thyroid) of a desiccated commercial preparation (No. 220005) from another firm. The next day the animal was dull and did not take its food; temperature 102⅜° F. Its weight was now 1680 grm. The dose was not repeated and the animal recovered in a few days.

Exp. 3. Rabbit; weight 1700 grm.; temperature 102° F.; was given 16 grm. (2½ grains = 5 grains of sheep's thyroid) of one of the earlier preparations of desiccated thyroid from a third firm. As no effect followed in twenty-four hours, the dose was repeated with an equally negative result. It should be noted that I had given 5 grain doses three times a day of this particular sample to man for the treatment of obesity, but without producing the desired effect; nor did diarrhœa, headache, pyrexia, etc., occur during a two weeks administration of this preparation.

Exp. 4. Large white rabbit was given 10 sugar-coated tablets of another unnumbered preparation (each tablet = 5 grains fresh gland) from the preceding firm. These tablets were finely pulverized and given as usual through the stomach tube. Result, death in twelve hours.

Exp. 5. Rabbit given three sugar-coated tablets obtained from a fourth firm. Result, death in four hours, preceded by paralysis of the hind extremities, cyanosis, some exophthalmos, rapid respiration, pulse about 280 per minute, two very slight general convulsions, just after the second of which the respiration ceased.

To rabbits, therefore, comparatively small amounts of either aqueous, stale thyroid extracts, or of commercial desiccated thyroid usually prove very toxic. I write "usually," for the toxicity of the different trade preparations varies very greatly. Thus the equivalent of 1 gramme of raw thyroid of a preparation by one firm will in many instances cause death in a rabbit, whereas many times that amount of another firm's preparation may produce slight symptoms only, which may completely disappear in one or two days. Different lots even from the same firm vary very much; some of the lots appearing to be but very slightly toxic, while others are excessively so.

(*b*) *Administration of thymus.* Wishing to see if identically the same results could not be produced in these very susceptible animals by means of other glands, meats, etc., I made a number of observations to determine this question. The following observation will indicate my results in general from these comparative administrations.

Obs. 4. A number of absolutely fresh lamb's thymus bodies—in many instances that of the calf was used also—were halved and separated into two portions, one of which was put aside for a known number of hours; the other was minced or made into bouillon, and fed to a rabbit, but in every instance with a negative result. After forty-eight hours or more the former portion was similarly prepared and fed to other rabbits. In some instances both the raw material or the concentrated bouillon from it produced death in from two to six hours, the animals exhibiting symptoms usually identical with those produced by very much smaller amounts of the stale thyroid. Usually, however, I found it necessary to keep the thymus glands on ice for a few days longer for marked toxicity to develop. I do not mean to give the impression that these thymus glands had become putrefied, in fact such was not the case, and were one to judge by their appearance and odor they would be considered still fresh enough for human consumption. Indeed, in my experiments with rabbits it has seemed to me that when a great amount of putrefaction has occurred in both the thymus and thyroid material, the bouillon, extracts, etc., appear to be somewhat less toxic. No doubt putrefactive processes alter the chemical structure of the chief toxic substance or substances.

The effects of administering the desiccated commercial preparations of thymus gland were not studied, but on one occasion I administered a dose of thirty-two grm. of a commercial nucleo-proteid * preparation prepared from thymus and other glands, to a strong healthy rabbit, without consequent ill effect.

(*c*) *Administration of meat extracts.* Closely related to the results obtained in this series of experiments on rabbits to which stale preparations of thymus and of thyroid were given, are the observations of Kemmerich,† Bogoslowsky ‡ and others. The former of these writers called attention to the fact that in addition to the salts, certain organic substances are present in meat extracts and are the cause of the cardio-excitant action that results from the ingestion of the meat extracts. Bogoslowsky, repeating Kemmerich's previous experiments, similarly found increase in the pulse rate and also a rise of temperature. Both fully agree that such extracts are excessively toxic to rabbits, usually producing death within a few hours. Kemmerich gave an extract equivalent to 1333 grm. of horse-flesh to a rabbit which rapidly began to exhibit the following symptoms: dullness; respiration increased from 120 to 180; pulse rose from 240 to 400 and became stronger; a stage of depression then supervened and the animal soon lost the power of maintaining the upright position, the hind legs appearing paralyzed; later violent dyspnœa and convulsions terminated the rabbit's existence. The pathological changes consisted of gastro-enteritis, hyperæmia, and many small gastro-intestinal ecchymoses. In other words, Kemmerich's rabbits all died with a chain of symptoms that are practically similar to those that may be produced in rabbits, and also in monkeys, by administering the stale or the dried thyroid material. The pathological changes found in all of my poisoned rabbits fully accord with those described by Kemmerich, but to satisfy myself fully of the correctness of the observations of these writers I repeated their experiments, using, however, ox-meat instead of horse-meat.

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* This nucleo-proteid preparation was prepared from various glands, of which the thyroid was one. Chemically, the preparation contained a considerable quantity of iodine.

† Kemmerich, Pflüger's *Arch.*, ii (1869), 49.

‡ Bogoslowsky, *Centralbl. f. med. Wissensch.*, 1871, 497.

Briefly stated, a quantity of absolutely fresh meat was procured from a neighboring slaughter-house and the extraction of four or five pounds of this finely minced meat begun within a few minutes after the death of the ox. As in the case of the raw thyroid material, half of the original quantity was put aside on ice for a number of days, then extracted by boiling and the bouillon given to rabbits. To sum up, the results in this series of experiments were as follows:

In no instance, after repeated trials, did I succeed in producing any toxic symptoms in rabbits by means of the bouillon from the *perfectly fresh* meat, even if the amount of the concentrated bouillon was equivalent to as much as five pounds of meat. After keeping the meat on ice for 10 days or preferably for 14 days, it being still sweet, the bouillon from it proved usually very toxic, causing death generally in from two to four hours, usually preceded by exophthalmos with or without nystagmus. Dyspnœa and slight convulsions also frequently occurred.

Similar extracts from good soup meat cut from carcasses that had hung fourteen days or more proved in some instances toxic, and in others not. In one experiment the extract was made from one pound of apparently good soup meat purchased at a third-rate butcher-shop and given to a rabbit, with the result that the animal died in less than an hour.

The symptoms exhibited by these animals poisoned by the stale-meat bouillons and the lesions found after death were similar to those presented by the animals poisoned with the thyroid preparations; but, for the meat to develop its full toxicity to rabbits, it was necessary to keep it very much longer than the thyroid or the thymus, not long enough, however, for a noticeable odor of putrefaction to develop.

The herbivorous horse and cow, according to Brinton,* may be brought to eat fish and flesh, but had the above-mentioned toxic substance developed in the flesh in considerable quantities, no doubt these animals would present the same degree of susceptibility to the meat extracts that is known to exist in the sheep.

Birds. Having fed a cock with large quantities of fresh thyroid gland, later with extract of fresh meat, and finally with extracts of stale meat, with absolutely negative results as regards the bodily weight and temperature, I administered to this bird large quantities of preparations of commercial thyroid, one gramme of which had produced fatal results in monkeys.

Obs. 1. Cock; weight 2520 grm.; temperature 108⅗° F; received daily the following preparations: 1st day, six 5-grain thyroid tablets † of Burroughs, Wellcome & Co.; 2nd day, six similar tablets; 3rd day, twelve ditto—weight 2500 grm.; temperature 108⅖° F. Feathers fall out readily; 4th day, 10 grm. of Parke, Davis & Co's desiccated thyroid; 5th day, 20 grm. ditto; 6th day, 30 grm. ditto; 8th day, 20 B. W. & Co's tablets (another lot); 9th day, 50 ditto; 10th day, 36 grm. of P. D. & Co's thyroid; 11th day, 32 grm. of Fairchild's thyroid; 12th day, 180 grm. of raw sheep's thyroids, three days old; 13th day, 60 grm. of putrid ox thyroid. Weight 2540 grm., temperature 108⅗° F.

The effect was thus negative. I have noted that the feathers of this bird fell out in great numbers, but as it was kept in a room maintained at a high temperature owing to the presence of monkeys, probably no special significance should be attached to this occurrence.

Cats. Owing to the difficulty met with in administering the dried preparations to these animals, but one experiment was performed, namely, I gave to a cat 100 B. W. & Co's thyroid tablets at one dose, but without disturbing the general health of the animal in the least.

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* Brinton, *Cyclopedia of Anat. and Physiol.*, v, 1859, p. 388.

† Chemical examination of small quantities of all the above commercial preparations, some remnants of which I still possess, reveals the presence of iodine, and presumably in the form of thyro-iodine of Baumann.

Dogs. In none of the numerous experiments upon normal dogs or upon previously splenectomized dogs could I succeed in producing the slightest sign of the intoxication. Even in the cases in which these animals were fed for periods of a week or more on nothing but sheep's thyroids, no alteration of the temperature occurred. Some of the animals, although thin and in very poor condition at the beginning of this diet, gained flesh rapidly and at the end of the experiment were in far better general condition than at the commencement.

Before any thyroid was administered to two of these dogs I exposed the thyroid lobes in each and carefully and quickly measured them. After the animals had been fed upon large quantities of fresh, stale and dried thyroid, and finally for a week upon nothing but stale thyroid, the dogs were anæsthetized, the lobes exposed and measured *in situ.* The latter measurements practically coincided with those at the beginning of the experiment, and thus no evident alteration in the size of these normal glands had followed the ingestion of the large quantities (300 to 600 grm.) of mixed thyroid.

This non-diminution in the size of the thyroids in these two dogs does not correspond to the results that Lanz * got by injecting rabbits and dogs with the thyroid juice. This observer states that owing to the extraordinarily inconstant relative size of the thyroid in rabbits he exposed and measured them before beginning the injections, and also during the period of their administration. Considering that possibly this repeated exposure of the glands may have given rise to errors, owing to the fact that the normal thyroid might, like the goitrous thyroid, diminish in size on exposure to the atmosphere, Lanz devised the following procedure to meet this objection: Two rabbits of the same litter were chosen and one was injected, but without previously exposing and measuring the thyroids of either animal. After a certain number of injections had been made both the rabbits were killed and their thyroids compared. The thyroid of the injected rabbit proved to be one-third smaller than that of the uninjected control animal.

It appears to me, however, that from this inconstant relative size of the thyroid, judging from my personal experience in both rabbits and dogs, the latter method is certainly open to greater objections than the former, provided the one initial aseptic operation is rapidly performed, care being taken to disturb the blood-vessels and lymphatics of the bodies as little as possible. When a number of exposures are made one can readily imagine that damage, either directly to the gland or to its blood supply, would be far more likely to occur, and result in some degree of atrophy of the gland.

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* Loc. cit.

Monkeys. Obs. 1. Bonnet monkey; weight 1260 grm.; temperature $101\frac{7}{8}$°; pulse 110. Given 8 grm. of sheep's thyroid that had been kept in the ice-box for twenty-four hours. It should be noted that on the previous day 35 grm. of this same lot had been given to another monkey without effect.

Result: The next morning, considerable depression; refused food, moderate diarrhœa, thirst; rapid and irregular heart's action, about 200 per minute; temperature 104° F. The dose was not repeated and the animal recovered in a few days, during which considerable emaciation occurred.

Obs. 2. Exp. 1. To a Java monkey, weighing 1358 grm., temperature $101\frac{1}{8}$ F., pulse about 96, that had been fed two weeks before on fresh gland with a negative result, was given 3 grm. of an unnumbered desiccated thyroid preparation from a certain firm. As this produced no effect by the next morning the animal was given 10 grm. more, and finally 12 grm. of the same preparation on the third morning. Having exhausted my supply of this particular preparation the experiment was discontinued and the animal observed for ten days. No ill effects resulted.

Exp. 2. To the same monkey six 5-grain tablets (each tablet = 5 grains of raw thyroid) of another firm were given. The next morning the temperature was 103° F., the pulse 124, and the animal not so lively as usual. It was then given six more tablets. On the following day the temperature was $103\frac{7}{8}$° F., the pulse 180; great thirst, considerable agitation, slight diarrhœa, emaciation, some exophthalmos, and marked nystagmus. No more tablets were given. 4th day, animal very ill, severe diarrhœa, great weakness, crouches all the time and seems unable to support itself in the upright position. Temperature 99° F. and pulse 140. 6th day, bloody diarrhœic stools, great weakness, and the hind legs apparently paralyzed. Temperature 98° F.; pulse about 260; respiration 68. The animal lived throughout the day and night in a partially comatose condition and died early on the following morning.

Autopsy. Marked gastro-enteritis accompanied by numerous small and large submucous and subperitoneal ecchymoses. Mesenteric glands swollen and much congested. Acute nephritis. Liver very friable and seat of many ecchymoses. Blood in portal vein clotted, while that in the inferior vena cava and cavities of the heart was still fluid. The thyroid gland was not enlarged and appeared unaltered microscopically.

Exp. 3. Java monkey; very lively and somewhat frightened. Weight 1995 grm., temperature 102⅕° F., pulse 180. Given 1 gramme of a thyroid powder from a third firm. On the next morning the animal was dejected, scarcely noticed its food, but was excessively thirsty, and had a very moderate diarrhœa. Temperature 105⅖° F.; pulse about 240. The dose was not repeated. On the third day this monkey was slightly better and took its food with some persuasion. The temperature was 105° F., and the pulse 160. The diarrhœa had disappeared. On the morning of the fourth day it was found lying on its side markedly agitated and squealing. Voluntary motion in the hind legs seemed to be lost and moderate exophthalmos and nystagmus were present. Shortly after several slight fits occurred and death ensued.

Autopsy (four hours after death). Weight of body 1725 grammes. Stomach, small intestines and mesentery the seat of a number of ecchymoses. Many minute hæmorrhages in the liver. Blood in the portal vein clotted; that in the heart and inferior vena cava still fluid. Thyroid body normal.

Exp. 4. A previous monkey, to which 3 grm. of this same preparation were given, exhibited within two hours considerable fibrillary muscular twitchings; slight exophthalmos; paralysis of the hind legs, apparently spastic in character, the knee jerks as well as sensation to slight pin pricks persisting; excessively irregular and rapid cardiac action; rapid and shallow respiration; rectal temperature 102⅖° F. (101⅖° F. normal for this animal). Several slight fits occurred, in one of which there was slight momentary opisthotonos, with considerable rigidity of the extended hind legs. Throughout, the animal seemed to be perfectly conscious, and when small pieces of bread were placed in its mouth tried to chew and swallow them but was unable to do so. In thirty minutes collapse, coma and death occurred.

Autopsy (five minutes after death). The usual hæmorrhagic areas in the intestines and liver, none in the stomach. The cerebral pia mater was deeply congested and also that of the cord. A peculiar tube-like thrombus about half an inch in length was present in the portal vein, but the blood in the other large veins and heart appeared abnormally fluid and of a laky tint.

Experiments were made in which a number of different lots from numerous firms gave practically similar results. I was unable to determine which firm's preparations were most toxic, for the toxicity of different lots from the same firm varied much, one lot proving excessively toxic, while another would produce toxic symptoms only after fairly large daily doses had been given for a period of several days.

All these toxic dried preparations produced pretty much the same pathological changes, but the symptoms induced by some of them in several monkeys differed in a few instances, one of which has been described in experiment 4.

Man. My experiences as to the effects of glycerine extracts and dried commercial thyroid upon man are essentially similar to those of other observers. Very severe intoxication has not been observed by me, probably because the effect of the thyroid was always carefully followed and the preparations stopped or decreased as soon as the earlier toxic symptoms appeared.

In many instances I gave portions of the preparations that had produced these toxic symptoms to monkeys, rabbits, and other animals, with the result that the monkeys and rabbits invariably exhibited severe signs of this intoxication.

Loss in weight, pyrexia and acceleration of the pulse occurred in man only when the dried preparations were given, never with the finely minced fresh raw thyroid material.

In man, as in the lower animals, it was also found that the toxic activity of all these dried preparations varied considerably. The susceptibility of the healthy individual seems to vary also, but not very markedly.

Obs. 1. An obese individual took two 5-grain B. W. & Co's tablets three times a day for three days with only an immaterial increase in the pulse rate. After four days had elapsed another lot from the same firm was given, and one of these tablets taken three times a day produced in four days severe headache, dizziness, a temperature of 102° F., pulse 96, insomnia for one night, and a moderate diarrhœa. Having completely recovered from the above by the end of a week, 4 grains (11 grains = 1 whole sheep's thyroid) of Parke, Davis & Co's dried thyroid were given three times a day for a period of three weeks. From this preparation the pulse increased to 88 in the first week, but fell to 80 in the second. One or two slight diarrhœic attacks occurred at the end of the first week, but none in the second. The temperature was not apparently affected. In the third week, on increasing the amount to 6 grains three times a day, the pulse rose to 90, the temperature to 101° F., and the occasional attacks of diarrhœa returned. 5-grain doses were then given and continued for three weeks longer. At the end of this time 16 pounds had been lost.

Other preparations of thyroid from this firm have not, however, been always so well borne, even one-grain doses three times a day giving rise to disagreeable symptoms in some people.

From Armour's thyroid in doses of three grains three times a day I have seen distinct toxic symptoms arise by the following morning in a fleshy female who had taken larger doses of a preparation by Fairchild without the occurrence of any ill effects. My average experience with this preparation is indicated by the following observation.

Obs. 2. A man weighing 191 pounds, who had previously taken eighteen 16-gramme doses of fresh raw thyroid without effect, was given 3 grains three times a day of Armour's thyroid. On the sixth day the pulse had risen from 78 to 94 and the temperature to 99° F. The above quantity was continued and at the end of the second week the pulse was 100, the temperature 101° F. Nausea, moderate headache and slight diarrhœa were complained of. The dose was reduced to 1 grain per day and the above symptoms disappeared. In three weeks the weight was lessened by twelve pounds.

Further observations in healthy men with these impure and complex substances would be merely repetitions of the above and are consequently omitted, for nothing would be emphasized by them except the general variability of the toxic properties of all of these dried preparations of thyroid.

C. *Thyroidism in diseased man.*

So far my observations as to the production of "thyroidism" by the thyroid material have included experiences only upon healthy men and other healthy animals. In reality, however, observation 1 of the following series first drew my personal attention to this subject. The remaining experiences that are described here are still more apropos.

Obs. 1. Adult man, 55 years of age, with paralysis agitans, took daily except Sundays about 8 grm. of finely minced fresh sheep's thyroid for a period of three weeks. The effect was absolutely negative. After one week had elapsed two 5-grain sugar-coated thyroid tablets of B. W. & Co. were given three times a day. On the morning of the third day the following symptoms of intoxication had developed: Excessive restlessness during the preceding night; severe dizziness and a sensation of tension over the forehead; severe dyspnœa; strong but irregular cardiac action, pulse 120 per minute, and temperature 103⅕° F. The thirst was insatiable, the general prostration considerable, and the face and neck were covered with a very prominent fiery red erythematous rash. In the afternoon of the same day a slight diarrhœa developed. The tablets having been discontinued, all these symptoms disappeared in the course of a few days and the patient rapidly gained in flesh and strength.

Obs. 2. A girl aged 18 years, with exophthalmic goitre. Pulse 125; temperature 99° F. Circumference of neck 13¼ inches. Left lobe of thyroid larger than right, and right eye more prominent than left. Tremor, insomnia, and numerous other symptoms including cramps and occasional attacks of tetany. Took for the first six days 6 grm. of fresh sheep's thyroid slightly cooked. Result, pulse 120, temperature 99°. Circumference of neck 13 inches (I had been previously informed by the patient that the struma often varied in size). 10 grm. of the finely minced raw gland were then given daily for twelve doses, after which the pulse was 110, the temperature 98⅕° F., and the measurement of the neck 12 inches. The weight of the patient had increased one pound. Previously to taking the fresh raw material this girl had been given one tablet (same lot as in Obs. 1) twice a day for three days, with the result that the tremor, cardiac symptoms, and general weakness decidedly increased. The left eye became more prominent and the habitual diarrhœic movements that usually occurred in the afternoon appeared in the morning also.

Obs. 3. Exophthalmic goitre, but with no evident enlargement of the thyroid gland. Pulse 136, temperature 98⅗°, weight 125 pounds. Took for one week except Sunday 10 grm. per day of fresh sheep's thyroid. Result, pulse 120, temperature 98⅗° F., weight 126 pounds. This man had been troubled with almost daily sudden attacks of diarrhœa previously to the beginning of the course of thyroid, and, therefore, for the first few days capsules of pepsin and subgallate of bismuth were given after each meal, the thyroid dose being taken three hours after breakfast. Under this treatment the diarrhœa quickly disappeared and did not reappear during the remainder of the time that the fresh gland was taken.

Obs. 4. Exophthalmic goitre; female, aged 28 years. Previously to coming under my charge she had taken fresh pigs' thyroid for a number of weeks in daily doses (amount unknown). After eight or ten weeks of this treatment in combination with baths and careful dieting she had gained several pounds in weight. The goitre had diminished somewhat at first, but later enlarged to its former size on stopping the treatment; no increase in the pulse rate or palpitation had been noticed. Some months later she had been given capsules of one of the dried American thyroid preparations. From the use of this preparation such a considerable loss in flesh, severe diarrhœa, marked increase in the general weakness, and severe tachycardia occurred that the preparation had to be discontinued.

This patient was given the perfectly fresh raw thymus (about 4 ounces) daily for a number of months with the result that the pulse rate decreased from 100 per minute to 84. The enlarged and firm thyroid became much softer, and diminished greatly (2 in.) in size. The absent menses returned. After a few doses of the pepsin and subgallate of bismuth the troublesome diarrhœa permanently disappeared; the exophthalmos was greatly lessened and von Graefe's sign entirely gone. The weight increased from 105 lbs. to 125 lbs. under this nutritious diet, and the strength is now such that the patient can walk four or five miles per day without fatigue.

Obs. 5. A man aged 20 years, with mild Graves' disease, had been taking twelve 5-grain thymus tablets of B. W. & Co. daily for two weeks. Without my knowledge he concluded to double the dose, and in three days, in addition to great thirst, dizziness, great restlessness, unsteadiness in gait, considerable dyspnœa and precordial oppression were developed. Accompanying the above were nausea, vomiting, diarrhœa and very severe general prostration. The face, arms and neck were covered with large urticarial wheals. The temperature rose to 102° F., the pulse to 150. The tablets were immediately stopped and most of the above disagreeable symptoms disappeared by the following morning, although the general depression and diarrhœa continued for several days. With very much larger doses of the fresh thymus no such symptoms reappeared in this case.

In one other instance I have seen the above symptoms occur during the administration of these thymus tablets even in doses of eighteen per day.

Obs. 6. Finally, I have had the opportunity of carefully examining and questioning an intelligent patient with myxœdema who took for about two months small doses of a certain commercial dried preparation without the least benefit; in fact, according to her statement, the various symptoms of the disease became more marked. The preparation in question produced in this case frequent attacks of severe diarrhœa, considerable acceleration of the pulse rate, headache, and much prostration. Another commercial preparation was substituted, and in consequence the myxœdematous symptoms rapidly improved, while the diarrhœa, headache, etc., disappeared and did not reappear unless large doses of this latter preparation were taken daily for several days. It should be noted that the doses of the respective preparations were the same, in this instance each tablet being equivalent to 5 grains of the raw gland.

About 30 grammes of the former preparation being in my possession, ten tablets were given daily to a thyroidless dog before the cachexia had developed. The appearance of the latter was certainly not delayed, on the contrary the dyspnoea and fits presented themselves earlier than usual (about 38 hours). On injecting an extract of these tablets into another thyroidless dog the cachexia appeared in 30 hours, and none of the symptoms lessened in severity from even repeated injections of this extract. The preparation undoubtedly contained very toxic substances, but whether any of the supposed active curative thyroid principle was present cannot be definitely stated, owing to the fact that the failure to relieve the symptoms in the above tests is not conclusive proof of its total absence. The important objections to these tests will be discussed more fully in Part III.

D. *Results and general considerations.*

The results of the foregoing observations conclusively demonstrate the two following main facts:

First, that the ingestion of very large amounts of finely minced fresh thyroid material gives rise to no intoxication whatever in man, monkeys, dogs, cats, rabbits and birds, provided the digestive processes in the said animals be performed with the usual normal rapidity.

Second, that the stale material or the preparations that have been made from the thyroid according to the methods usually in vogue may or may not produce in certain animals the symptoms of an intoxication. In many instances the symptoms may follow even comparatively small doses.

In normal men, monkeys, dogs, cats, rabbits and birds I have never been able to produce by means of the fresh thyroid material a rise of temperature, acceleration of the pulse either shortly after the ingestion of the dose or on the following day, loss in weight except in rabbits (?), agitation, general prostration, or exophthalmos. Further, none of the above normal individuals exhibited any perceptible enlargement of the thyroid gland, nor did accurate comparative measurements of the lobes before and after the ingestion of the thyroid indicate that any discernible diminution in size had occurred.

Carnivorous animals (dogs and cats) and birds (cocks) appear to be insusceptible to the action of the toxic preparations even in enormous doses; but man, monkeys, and especially rabbits, speedily manifest the various symptoms in greater or less prominence. This marked difference was not surprising in the least, for many previous investigators of poisonous meat, sausage, cheese, and other ptomaine-containing foodstuffs, note the frequent immunity of carnivorous animals from these poisons. The natural propensity of cats, and of dogs in particular, for all kinds of carrion and sundry bits of putrefied food is also well known and needs no comment further than the remark that no ill effects follow numerous meals of this character. Boehm * reports that a dog was given a large bowl full of poisonous cheese, all of which he ate, but no toxic symptoms followed. Chickens and sparrows, according to Gaertner,† are also insusceptible to toxic meat, and other observers have reached a similar conclusion. In dogs, however, as has been stated elsewhere, Lanz noticed some acceleration of the pulse rate, but Edmunds ‡ produced no effect by feeding dogs on sheep's thyroids.

All the dogs and other animals to which I gave the dried commercial thyroid preparations exhibited great thirst, but as these preparations are often one-half chloride of sodium, considerable thirst is what one should expect. Great thirst consequently indicates nothing that could be definitely attributed to the special action of the toxins. As regards the emaciation noticed by Lanz I may add that at the beginning of the experiment the majority of my dogs were very thin and poorly nourished, but while taking large quantities of all kinds of thyroid material and receiving excellent food, they rapidly fattened and continued to do so while under observation. If these animals had been kept housed for a number of months and fed on thyroid they naturally would have ultimately fallen into a poor condition; and so would other dogs if deprived of out-door exercise and air, no matter

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* Boehm, Ziemssen's Handbuch, Bd. xv, p. 249.

† Gaertner quoted by Oestertag, Fleischbeschau. Stuttgart, 1892.

‡ Edmunds, loc. cit.

how well fed the animals may be. Normal carnivorous animals, in fact, are not at all suitable subjects for such experiments if one has to keep them in confinement for a long time. Most of the toxic thyroid preparations do cause considerable loss in weight in rabbits, monkeys, and in man. It is conceivable that the fresh material may do this also if gastro-intestinal fermentation occur and the altered thyroid products become absorbed. This latter circumstance, I think, very probably explains many of the experiences in which clinicians have observed mild or severe toxic symptoms to follow the ingestion of fresh raw material. This marked katabolic effect of the substances contained in these toxic preparations is not, however, specific for the thyroid only. Preparations of other ductless glands are liable to produce a very similar intoxication accompanied by emaciation. Many tox-albumoses, toxic proteids and ptomaines from poisonous meat and sausage, fish, cheese and other food-stuffs, usually produce a similar marked emaciation, as is clearly shown in the well-known works of Bollinger, Brieger, Backlisch, Müller, Panum, Marino-Zucco, and many other observers.

Generally speaking the intoxications produced by all of these poisons from altered food-stuffs resemble one another in some respects, that is, certain symptoms appear to be more or less common to them all, while other symptoms are often essentially different. My experiences with the toxic thyroid material teach that this statement is valid for the clinical picture of this variety of intoxication also. Neither in the acute nor in the chronic forms of " thyroidism " are the various individual symptoms always uniform as a whole. Thus one commercial preparation seems to affect the gastro-intestinal canal chiefly; another preparation, even from the same firm, appears to suddenly overwhelm the muscles; others act upon the central nervous system of monkeys and of man also, so that either a spastic or a flaccid form of motor paresis or of paralysis may occur. Sometimes exophthalmos is prominent; at others no such condition is visible. All of these toxic substances seem to profoundly alter the blood.

In monkeys the majority of thyroid preparations produce a rise of temperature; on the other hand some of them in concentrated doses produce just the opposite effect, a fall of 4° F. below the normal having been witnessed by me. In some cases dyspnœic symptoms are marked; with other preparations the respiratory rhythm may from the beginning become progressively slower and finally cease, the excessively rapid heart-beat continuing for a short time afterward. In some of my monkeys no tremor or restlessness was noticeable; several others, however, exhibited this agitation very prominently. Acceleration of the pulse appeared to be produced by all the toxic thyroid preparations that were tested by me.

An analysis of these contradictory physiological effects appears to indicate that very probably these toxic preparations contain frequently different toxins or at times a combination of toxic substances. In certain preparations one variety of toxin or toxins predominates, in others a toxin of a slightly different nature may occur and superadd its effects to those produced by the other toxins which possibly enter into the composition of the preparations in question.

From Observation 6 under C, and from practically similar ones of other clinical observers, I am warranted also in stating that the toxic action of some of these dried thyroid preparations exceeds their palliative action in myxœdema; in fact certain symptoms of the latter disease may be made worse thereby, and no palliation at all as regards certain others may follow. This fact does not, however, necessarily prove that none of the supposed thyroid principle, which most observers, as far as I am aware, concede to be present in the thyroid gland, is contained in the preparation; for until this principle is obtained in a pure state, the above clinical test of the complex crude product only demonstrates that either the susceptibility of the individual is very great or that the toxins are particularly active, or both. If a certain toxin be present in considerable amount, this very toxin may possibly nullify or partly counteract any effect resulting from the beneficial thyroid principle or principles.

In reality many of the symptoms produced by these toxins from the thyroid material and from other toxic animal material also, show a remarkable and suggestive similarity to those composing the clinical picture of the experimental acute cachexia thyreopriva. Many of the symptoms that are exhibited by intoxicated men, monkeys and rabbits on the one hand, and by thyroidless carnivora in the early stages of the acute cachexia on the other, bear also a striking similarity to some of the most prominent symptoms and complications of exophthalmic goitre. Thus in all three conditions the central nervous system manifests its over-activity by fibrillary muscular tremor or twitching, tetany, etc. The contractions of individual muscles or groups of muscles so common in the intoxications and in the cachexia, frequently occur also in exophthalmic goitre. Rigidity, contracture, and a variety of spastic paralysis (Marie, Charcot) are also symptoms which may be included in the symptomatology of each. The rapid emaciation, the prostration and the weakness occurring with the intoxications, with the acute cachexia, and usually with Graves' disease. need no comment. A rise of temperature is frequent in exophthalmic goitre, is usual in the intoxications; and on the first few days following the thyroidectomy, during the acme of the muscular twitchings in the cachexia, the temperature often rises 4° or 5° above the normal (Hertzen, Ughetti, Alonzo, Rogowitsch, Horsley). Considerable acceleration of the pulse is also invariable, I find, in the early stages of the acute cachexia in dogs and monkeys. Slight exophthalmos * in the experimental cachexia is rarer, but does occur under circumstances which I shall describe in the following division of this paper. Some of the toxins from stale meat, thymus, thyroid, etc., frequently produce this symptom to a marked degree in rabbits and in monkeys, although in normal dogs I have utterly failed to produce this effect when the toxic substances were given by the stomach.

In exophthalmic goitre my own rather limited experiences and the very numerous clinical reports of others clearly indicate that certain symptoms are very materially increased by these toxin-containing thyroid preparations, whereas the absolutely fresh raw material produces, according to my experience, no such result.

The advocates of the theory that a too active thyroid produces Graves' disease, while admitting that possibly some of the symptoms

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* Edmunds has also noticed the occurrence of exophthalmos after removal of the thyroid.

following the ingestion of the toxic thyroid preparations may be due to chance ptomaines, may raise the objection that the supposed thyroid principle is of the nature of an enzyme (White and Davies, Notkine), and that the thyroid gland contains immediately after its removal the zymogen only of this enzyme. This state of affairs is well known to exist in the case of the pancreas, which after removal develops digestive properties only after a certain period of time has elapsed. The nature of this generally conceded thyroid principle is, however, very obscure, but were this enzyme the active principle, toxic in character, one might reasonably suppose that it would under varying circumstances preserve its toxicity, that is to say, if this special substance be obtained in a pure form it should continue to be toxic as before, though possibly the toxic symptoms that it produces might differ somewhat from those produced by the crude toxic material. I have consequently endeavored (Part III) to isolate from the various preparations of thyroid sundry substances in a pure state which have been given to normal and also to totally thyroidless or partially thyroidless animals. It is needless to say that the results in this direction do not in the least disagree with those obtained in the present part of this paper.

I reiterate that the fresh raw thyroid material shows not the least tendency to produce intoxication; that I have not found that the symptoms of exophthalmic goitre are increased by its ingestion, providing of course that no toxins develop in it during gastro-intestinal digestion; that a certain toxic substance or group of substances may or may not be present in the stale thyroid material and other animal material, which substances may produce in certain animals symptoms somewhat analogous to many of those usually met with in exophthalmic goitre. Though both experimental and clinical evidence demonstrates the fact that these analogous symptoms are produced by the toxic thyroid preparations, this fact cannot be logically or justly brought forward even as auxiliary proof to support the theory of those (Moebius and others) claiming that an over-active thyroid is responsible for the various symptoms manifested in Graves' disease.

PART II. THE INFLUENCE OF FOOD UPON THE APPEARANCE OF THE ACUTE CACHEXIA THYROIDECTOMICA.

A. *Historical introduction.*

Munk[*] having observed that a diet composed of lumps of meat hastened the death of thyroidless dogs, Breisacher,[†] a pupil of Munk, investigated this relationship and found that a certain number of thyroidless or partially thyroidless animals could be kept alive if fed with milk or with milk and eggs only. If raw meat or soup were given to these apparently normal animals the characteristic symptoms of the cachexia quickly developed. Meat carefully extracted by long boiling did not produce this effect. In animals in whom ⅕ or ⅐ of the thyroid had been left the bouillon was well borne, but as soon as the remaining portion of the gland was removed the symptoms of the cachexia quickly appeared, providing the raw meat or bouillon were given. If the meat salts were mixed with the milk the action was occasionally similar to that of the bouillon. In certain thyroidless dogs, although the tonic and clonic muscular contractions and dyspnœic attacks had appeared, these symptoms disappeared if the milk diet was continued. De Quervain,[‡] and later Rosenblatt,[§] also conclude that the progress of the cachexia is milder if the animals are fed on milk only. Masoin,[‖] on the other hand, claims that this pretended influence of milk diet upon the cachexia does not exist and that the duty of the thyroid gland is to nullify various toxins that are produced by the organism, which toxins accumulate in the blood and give rise to the convulsions, etc. The antitoxic function of the thyroid, previously emphasized by Colzi, Rogowitsch, Fano and Zanda, Schiff, Gley, and many others, is insisted upon by Masoin, and the intoxication is thus classed with the auto-intoxications.

The experimental evidence as to the influence of different food-stuffs, consequently, is by no means clear, and, as Ewald has remarked apropos of Breisacher's results, it is by no means definitely settled whether it is the disturbance of the metabolic processes in the tissues of thyroidless animals which directly produces the cachexia, or whether it is the components of various food-stuffs which are directly toxic. The mere administration of various kinds of food does not, as Ewald states, definitely settle this question, but from such experiments one can gain a fairly definite knowledge of the relative susceptibility of certain animals before and after thyroidectomy to the constituents of certain foods, provided certain factors, which if present are liable to give rise to very erroneous conclusions, be duly taken into consideration.

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[*] Munk, *Berl. klin. Wochenschr.*, 1889.

[†] Breisacher, *Arch. f. Anat. u. Physiol.*, Physiol. Abtheil., 1890, 509.

[‡] de Quervain, Virchow's *Archiv*, cxxx (1893), 504.

[§] Rosenblatt, *Arch. d. scien. biolog.*, St. Petersburg, iii, No. 1, 1894.

[‖] Masoin, Paul, *Arch. de phys. norm. et path.*, 5 sér. vi (1894), 291.

Briefly summed up, these factors which so seriously complicate the study of this problem are nothing more than the entire series of those "conditioning circumstances" (Horsley) upon which, as Horsley and others point out, the effects of thyroidectomy depend. Thus of all classes of animals the thyroidectomized carnivora, dogs, cats and foxes, exhibit the severest and most rapidly developed cachexia, death usually occurring between the third and fourth day in a comatose state (Wagner). According to Horsley the average duration of the neurotic, or first stage of the cachexia, is from one to two weeks in dogs; from one to three weeks in monkeys; young dogs and young monkeys alike dying in this stage. Elderly dogs merely exhibit symptoms of indolence and incapacity, indicating that the activity of the gland appears to be most active when tissue metabolism is most active. Under "other conditioning circumstances" Horsley * further dwells upon the important influence of a state of low nutrition before thyroidectomy, and upon the influence of the surrounding temperature upon thyroidless animals.

Possible residual thyroid tissue and the existence of accessory glands (Piana, Wagner, Fuhr, Ewald, Carle, Autokratow, Gley and Phisalix) and para-thyroids (Sandstroem, Baber, Gley, Kohn, Edmunds), may exert so great an influence that either the cachexia may not appear at all in dogs, or after a few very mild symptoms have been exhibited all signs of it may entirely disappear.

The operation of removing the thyroid lobes of the dog is an exceedingly simple one, but if the lobes be shelled from their capsules, as is usually done, one or other of the small para-thyroid bodies may be, as Gley has pointed out, unintentionally left behind. Should two or more be left, very slight or even none of the symptoms of the cachexia may sometimes occur, a personal experience which agrees with that of Gley. In certain young dogs both thyroid lobes, the para-thyroid bodies and any neighboring discernible accessory glands may be removed, nevertheless the results may be the same, or after the expiration of from five to ten days the severer nervous accompaniments of cachexia may develop, frequently with astonishing rapidity. In the first convulsive attack the dog may die, or several attacks of dyspnœa, convulsions, etc., which progressively increase in severity, may succeed one another during the

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* Horsley, loc. cit.

next few days, the dogs ultimately dying often during a severe convulsive attack. Other dogs and also cats may exhibit only moderate fibrillary twitchings and stiffness of the hind legs for numbers of weeks or even months, becoming to all appearances myxœdematous, and finally die in that condition. In a few others, after slight symptoms have existed for a number of weeks, they apparently subside completely and the dogs seem normal. The two latter courses, it is almost needless to remark, are generally exceptional.

According to my experience the vast majority of young dogs in good condition will, when they are fed on ordinary raw meat both before and after the operation, begin to manifest the symptoms of an impending dyspnœic attack within 52 to 54 hours after the total excision of the thyroid, and usually die in convulsions very shortly after the dyspnœic attacks have begun. In such dogs I consider that it may be justly assumed that for all practical purposes a total thyroidectomy had been performed. Very evidently none of the accessory glands that possibly remained were sufficiently active for the performance of much of the function of the thyroid. Nevertheless a careful dissection of the hyoid and aortic regions of some of the apparently thyroidless dogs, and of a large number of normal dogs also, frequently resulted in my finding one or more tiny, pale reddish globules from 5 to 1 mm. in diameter, which under the microscope presented a structure identical with that of the para-thyroid body, or parts of a continuous section appeared similar to that body (Plate XV, Fig. 2), while other fields of the same section had the appearance of the true thyroid structure. In all my young dogs and also in two cats, in which the cachexia developed mildly and not until four or five days after the operation, these tiny accessory bodies were invariably found. In some of the dogs additional accessory bodies of greater dimensions (2 to 8 mm.), such as Fuhr, Piana and others describe, occasionally accompanied the smaller ones. Two dogs that survived for sixteen days possessed each three small aortic bodies only about 1 mm. each in diameter, and in both dogs I found on the right side of the root of the neck just above the sternum a small round accessory body about 1.5 mm. in diameter, similar in structure in every way to the normal thyroid body.

The minuter aortic accessory bodies, often embedded in fat, are exceedingly difficult to find, and during a careful dissection by even the most experienced are very liable to be passed aside as bits of fat. Small bodies, apparently thymus remains, will also be met with and macroscopically may easily mislead. My own plan was to trust only to the microscope and examine everything that appeared to be the least suspicious. In numbers of instances where this latter plan has been neglected by observers and judgment pronounced as to the absolute absence of those accessory bodies, I feel very confident that many erroneous statements have been made.* Too much stress, therefore, cannot in my opinion be laid on the importance of this factor in influencing both the rapidity of the appearance and the degree of the cachexia in dogs and cats.

It is easily seen that in consequence of this latter complicating conditioning circumstance, numerous experiments, many careful and tedious dissections, and much laborious microscopic examination of suspected bits of tissue had to be performed before I could obtain a really definite idea as to the average number of hours which usually elapsed between the operation and the beginning of the dyspnœic attack in the totally thyroidless dog. The average that was given above was obtained from fifteen young dogs of about six months to one year in age, and the animals were fed both before and after the thyroidectomy on raw meat.

In studying this problem naturally the contents of the intestine at the time of the operation had to be regarded as a possible source of origin for sundry ptomaines, etc., whose additional action might complicate the results. Every operation was thus performed not earlier than twenty-four hours after the last meal, and in addition precautions were taken with a number of animals to feed them on bread and water for at least two days before performing the thyroidectomy. This latter precaution, I may add, did not, however, appear to influence the average time of development one way or the other. But if the dogs were fed on bread and water alone for several weeks before the operation the general condition of the dogs was liable to become so poor that the cachexia frequently developed rather more rapidly. A preliminary diet containing a greater amount of albuminous food is thus to be preferred.

My experience with totally thyroidless monkeys has not been large, but within twenty-four hours after the operation I have noticed, even in partially thyroidectomized monkeys, moderate fibrillary muscular twitchings, sluggishness, and a very marked increase in the susceptibility to cold; the animals constantly keeping on the day after the operation close to the steam heating pipes, while before the operation no proclivity for the hot pipes had been exhibited by the monkeys, which were kept in a room always carefully maintained at a uniform temperature.

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* In herbivorous animals also (calf and lamb) a number of these small bodies are usually present, and possibly their presence accounts in a great measure for the long period of time required by these animals to fully develop the symptoms of the cachexia.

My general mode of procedure was essentially the same as has been pursued by Breisacher and others and needs no further comment.

Comparative observations were thus made in which perfectly fresh raw meat, extracted meat and special extracts from fresh and from stale meat were used. Fresh and also dried toxic thyroid material were given to certain animals and the resultant effects noted. Comparative experiments with undoubted fresh milk were naturally out of the question in a large city to which the milk is brought often from a distance of many miles.

B. *Experiments.*

1. *Dogs.* In the following table (p. 192) I have tabulated certain of my trustworthy experiments on the dog in the form of series. In each series I report with three exceptions only those animals in which not the slightest trace of residual thyroid, para-thyroid, or accessory thyroid tissues could be found after a most careful dissection and a microscopic examination of all tiny bits of possibly suspicious tissue. In series XIII, Dog 3, I was inclined at first to question the actual absence of one or more of the minuter accessory bodies, but later experiments in which certain thymus extractives were injected lead me to attribute the short delay of the dyspnœa to the effects of the thymus diet.

Necessarily from the nature of the experiments the figures are but approximate, indicating in a general way the average number of hours that elapse after total thyroidectomy before the average young dog in an average condition begins to manifest the dyspnœic attack.

Numerous necessary exclusions have unfortunately caused the number of trustworthy observations in some of the series to diminish considerably, and in consequence it may be objected that some of the averages include too small a number of definite uncomplicated observations, especially as the averages differ not by days but by hours only. Granting the validity of this objection and also the possibility that the actual figures of some of the averages of the series might be slightly different had not so many of my dogs (60 per cent.) been provided with accessory bodies, I nevertheless offer these results for what they are worth.

INFLUENCE OF FOOD UPON THYROIDECTOMIZED DOGS.

Series.	Number of dog.	DIET.		Dyspnœa began.	Survived hours.	Resid. or acces. thyroid.	Number of observations.	Max. hours before dyspnœa.	Min. hours before dyspnœa.	Average time of series in hours.	REMARKS.
		Before operation.	After operation.								
I.	1	Raw meat.	Raw meat.	52	53	None.	15	56	47	53	
II.	3	Bread.	Raw meat.	51	52	None.	6	54	49	52	Died in second fit.
III.	4	Bread.	Milk.	48	54	Small aortic.	4	58	48	53	Severe diarrhœa.
IV.	4 8	Milk. Milk.	Milk. Milk.	50 45	58 48	None. None.	8	60	45	53	Poor milk for No. 8.
V.	2	Bread.	Bread.	64	69	None.	6	78	52	65	
VI.	2	Bread.	Boiled meat.	52	56	None.	4	60	52	56	
VII.	1	Bread.	Fresh raw meat.	70	71	None.	3	70	53	64	Died in first fit.
VIII.	2	Raw meat.	Bread.	58	66	None.	3	76	58	65	
IX.	1	Raw meat.	Fresh raw meat.	65	72	None.	3	65	58	63	
X.	7	Raw meat and dried thyroid.	Bread and dried thyroid.	38	42	None.	8	54	38	43	Diarrhœa in nearly all these dogs.
XI.	4	Raw meat.	Fresh raw thyroid.	96	113	One small aortic.	4	96	58	68	No. 4 also repeatedly injected with thyroid after beginning of fits.
XII.	2	Bread.	Fresh thyroid.	92	97	None.	3	92	60	76	
XIII.	3	Raw meat.	Bread & raw thymus.	88	96	None.	3	88	62	75	
XIV.	4	Raw meat.	Stale raw thyroid.	45	47	None.	4	53	45	48	
XV.	3	Boiled meat.	Meat bouillon & bread.	54	60	None.	3	56	47	52	
XVI.	1	Raw meat.	Milk.	49	54	....	2	50	49	50	No autopsy on dog 1. Milk of excellent quality.
XVII.	2	Raw meat.	Meat stew.	96	125	Three aortic.	2	....	53	53	Spleen removed 23 days previously.
XVIII	1	Raw meat.	Fresh thyroid, boiled meat.	58	66	None.	2	58	53	56	Acceleration of pulse, elevation of temperature, and emaciation occurred in all these dogs. The wounds remained aseptic.

Disregarding the diet of the animals previous to the operation and averaging the various series, one finds that the influence of the after-diet appears to be as follows: raw meat, 53 hours; fresh raw meat, 63 hours 30 minutes; bread and soup, 52 hours; ordinary meat extracted by boiling, 56 hours; city milk (fair quality), 52 hours; wheat bread, 65 hours; meat stew and fresh raw thyroid, 56 hours; fresh thymus, 75 hours; toxic thyroid material, 45 hours 30 minutes; fresh thyroid, 76 hours.

These averages would appear at first sight to indicate that both the kind and the quality of the diet do to a very slight degree influence the average time of appearance of the dyspnœic attacks in totally thyroidless dogs, but the differences are entirely too small in my estimation for one to positively assert the absolute correctness of the indication. The differences are certainly not as great as the observations of Breisacher would lead one to expect, practically no difference between the effect of ordinary raw meat and that of milk appearing to exist in the totally thyroidless dogs. In fact no matter what the diet may be, the totally thyroidless young dog very quickly develops one or more of the symptoms of the cachexia and dies usually within a few days after the performance of the thyroidectomy. Even if large amounts of fresh thyroid material be included in the diet the appearance of the cachexia is evidently but slightly delayed, although some of the symptoms are frequently modified remarkably in severity. Nevertheless I have not been able by this means to prolong the lives of totally thyroidless young dogs longer than 97 hours after the thyroidectomy. Possibly this period might have been extended had the minced thyroid been successfully introduced into the stomachs of the dogs by artificial means, for I have noticed that generally by the second or the third day food of all kinds is usually left untouched, water alone being voluntarily swallowed in small quantities. On attempting to feed the dogs through the stomach tube, even after cocainizing the fauces, it was found that, although very small amounts of milk and thyroid had been injected, practically the whole amount was almost immediately vomited. Under these circumstances and with the rapidly progressive emaciation and general weakness, death of course quickly followed.

If the totally thyroidless dogs were fed with large quantities of fresh thymus gland or with concentrated bouillon from the fresh gland, a somewhat similar though less marked effect both upon the time of appearance of the dyspnœa and upon the severity of the symptoms in general appeared to occur. In an earlier series of experiments, to which I have referred elsewhere,* I did not notice this favorable influence of the thymus when fed to the thyroidless dogs; but on repeating the earlier experiments and using much larger quantities of either the fresh thymus or of the concentrated thymus bouillon, I got the very appreciable beneficial effects to which I now call attention.

The dried commercial thyroid preparations and dried thyroid prepared by myself did not appear to delay the cachexia in the least, even though the doses of the dried thyroid were increased to 30 grammes (equivalent to about 100 grammes of the raw thyroid) per day. Judging from the average of the figures obtained in the eight dogs mentioned in the table, the ingestion of these preparations seemed on the contrary to hasten slightly the severer symptoms of the cachexia. It should be stated, however, that small portions of all of the dried preparations used in this series of dogs had been given to normal rabbits or monkeys and all had proved very toxic to both the latter animals. Two of the preparations contained the active palliative thyroid substance also, for by chemical means the substances that appeared to be toxic when taken by the mouth were removed, and the active palliative substances were injected subcutaneously into totally thyroidless dogs with very favorable results.

According to my experience, the cachexia so quickly overwhelms the completely thyroidless young dog, whether fed on milk, bread, or even fresh thyroid, that the comparative effects of different foods cannot be accurately tested upon the same dog, and the plan pursued by Breisacher of partial thyroidectomy would appear to be the one to be followed if the dog is to be the animal chosen for the experiment. Although it is open to many serious objections, this method was employed in eight dogs, but I got nothing that could be called an unquestionably positive result. The method was as follows: In seven young anæsthetized dogs the whole of the right thyroid along with its capsule was removed. On the left side about the upper fourth or fifth was left, none of the large superior vessels being interfered with. In the eighth dog the left lower fourth only of the thyroid body was left, precautions being taken to disturb its vascular supply as little as possible.

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* *Med. Record*, New York, June 15, 1895.

Four of these partially thyroidless dogs did not appear to differ in the least from normal dogs, and from the second day after the operation were fed on raw meat for three to four weeks. During this period no symptoms of the cachexia were manifested. Of the remaining four, the three in which about the upper fifth of the left lobe had been left presented evident though slight tremor in the rear extremities by the second day, and in running about appeared to use the hind legs somewhat clumsily. On the third morning these symptoms could not be detected and two of these dogs were put upon a diet of stale but good meat. As no further symptoms occurred, after two weeks of this diet, the two dogs were used for other experiments. To the third dog were given, besides his bread and water diet, 20 grm. of a dried commercial thyroid preparation, 1 grm. of which had proved fatal to a rabbit. On the next morning the temperature of this dog had risen 1° F. and the pulse rate had increased by thirty beats. In the hind legs the tremors had reappeared and a slight degree of stiffness and awkwardness in gait was apparent. Having but 10 grm. left of this thyroid preparation, this amount was administered and the dog carefully examined on the following morning. The temperature and the pulse were the same as on the previous day and the tremor and tetany were also still prominent. The bread diet was changed to raw meat and the dog observed for three weeks. On the seventh day the temperature and pulse were the same as before the operation, and during the rest of the time the animal did not appear to differ from a normal dog.

The dog in which the lower fourth of the left thyroid lobe was left presented by the third day considerable tremor, tetany and emaciation. On the fifth day the dog appeared better, took a little bread and a small amount of thoroughly boiled milk. On the ninth day (afternoon) the wound was found perfectly healed, and but for a just perceptible occasional fibrillary twitching in some of the muscles of the hind extremities the animal did not seem to differ from a normal one. On the tenth morning it was found in a fit from which it recovered in a few minutes, exhibiting, however, the rapid respiration and dyspnœa. After three similar fits during the day and probably more during the night the animal died on the eleventh day, in spite of several large subcutaneous doses of a commercial thyroid elixir. On dissecting out the remnant of residual gland it was found to have undergone almost complete atrophy, due doubtless to insufficient blood supply. The residual thyroid in the preceding seven dogs had on the contrary become considerably hypertrophied, the blood supply apparently not having been interfered with.

In four of the dogs, therefore, not enough of the thyroid had been removed to even temporarily disturb the physiological equilibrium. In two of the remaining dogs it was evident that though some disturbance had followed the partial excision of the gland, the vulnerability of the two animals was not sufficiently diminished to show the possible effects resulting from stale meat diet. A slight susceptibility to the toxic substances contained in the dry thyroid preparation would appear to have been produced in the seventh dog, but one cannot positively assert that the slight temporary return of the symptoms was not simply a coincidence dependent upon a temporary disturbance of the activity of the residual thyroid tissue. At any rate it was evident that the functional activity of the residual thyroid had been sufficiently recovered by the seventh day for the metabolism of this dog to proceed in a practically normal manner.

In the eighth dog, though the progress of the cachexia was slower, the final effect was the same as that following total thyroidectomy. Thus, in spite of the bread and milk diet the fits occurred and the cachexia progressed just as one frequently sees it in dogs in which both thyroid lobes have been removed, but in which a certain amount of accessory thyroid tissue has been unavoidably left.

It is well known that it is extremely common for dogs in which a small amount of residual or accessory thyroid tissue has been left, to have sundry dyspnœic and convulsive attacks, between which the dogs may seem normal. Consequently, if the milk diet is changed to one of raw meat, and the dogs, like those of Breisacher, have an attack after one or two days, it is absolutely impossible to decide whether the ingested constituents of the meat really induced the attack or not, for it is readily conceivable that the dogs might have exhibited the symptoms had no meat at all been given. One very definite fact obtained, however, is that the cachexia develops in the totally thyroidless dog just as readily with the milk diet as with the meat diet.

2. *Monkeys.* In monkeys the results appeared to be slightly more definite, for shortly after the partial thyroidectomy these animals appeared to become more sensitive to moderate doses of certain commercial meat extracts, as the following experiment indicates.

A large normal Java monkey was given 35 grm. of a commercial meat extract. This produced a moderate diarrhœa which disappeared in twenty-four hours, but no other disturbance could be detected. On the sixth day 20 grm. of the same boiled meat extract were given, but on this occasion no diarrhœa resulted. After the expiration of two days the dose of 20 grm. was repeated and was again well borne by the monkey.

Partial thyroidectomy was then performed, approximately the upper fourth of the left thyroid lobe being left, and the monkey confined to a diet of bread, fruit and water. On the morning after the operation occasional fibrillary twitchings were distinctly present in many muscles, the temperature had risen from 101⅜° F. to 103° F., and the heart-beat from 100 to 156. On the second day the monkey appeared to be chilly and continually kept close to the steam heating apparatus and could not be enticed away from it even by a banana, of which the monkey was very fond. Pulse 186; temp. 104° F. The aseptic dressing was removed and, after the small wound had been found to be in perfect condition, was replaced.

By the fifth day the appearance of chilliness had disappeared, the temperature had become 102° F. and the pulse 112. On the ninth day the wound was re-examined and found to be completely healed. The temperature was 99⅜° F., the pulse 96, and the monkey seemed as lively as before the operation. On the tenth morning 15 grm. of a meat extract concentrated from perfectly fresh ox muscle was given to this monkey, but without affecting the animal in the least.

Two days later at 11.30 A. M. 10 grm. of commercial meat extract, the same kind that had been given to the monkey before the partial thyroidectomy, which had been sterilized by boiling and kept in a sealed tube from the day on which the original package was opened, was administered by means of the stomach tube and the following effects resulted: After the expiration of about one hour the monkey became quite restless and had one large watery movement. In about two hours and a half the animal exhibited still greater restlessness, and the temperature had risen from 99⅜° F. to 101° F., and the heart-beat to 140 per minute. At this time a slight though easily discernible degree of exophthalmos accompanied by horizontal nystagmus was apparent. By six o'clock in the afternoon a considerable degree of fibrillary tremor was present, but the transient exophthalmos and nystagmus had almost disappeared. On the following morning the temperature was 103° F., the pulse 180 per minute; the fibrillary tremor still marked, but the exophthalmos and nystagmus had entirely disappeared. The animal appeared to be again chilly, and when removed from the neighborhood of the steam pipes it immediately returned, exhibiting considerable awkwardness and stiffness in its movements. Throughout the day the thirst was very pronounced, although the diarrhœa had practically disappeared. On the afternoon of the second day the pulse was 100, the temperature 100⅜°, and all the symptoms of chilliness and the fibrillary twitching had disappeared. On the fourth day after the animal had apparently recovered from the effects of the above dose, a similar amount of the same meat extract was given and the above-mentioned symptoms promptly returned, but were not quite so pronounced, and no nystagmus could be detected. On the third attempt to reproduce the preceding symptoms in this monkey, in spite of the fact that 15 grm. of the meat extract were given, the exophthalmos did not recur; the pulse and the temperature, however, increased slightly, and the appearance of chilliness, although prominent on the morning after the dose had been given, disappeared during the afternoon of that day.

Five weeks after the operation another 15 grm. dose of the meat extract was given to this monkey, which had apparently become perfectly normal again, and except for a temporary increase of twenty beats in the pulse-rate and a little transient diarrhœa, no effect followed. At this date the residual portion of the thyroid gland could be distinctly felt and was evidently much increased in bulk, and judging from the experiences of Wagner, Horsley, von Eiselsberg, Halsted and Breisacher, I conclude that a true hypertrophy of its glandular elements had probably occurred.*

Thus, as had been previously anticipated, when sufficient functional activity of the residual thyroid had been regained, the increased susceptibility of the monkey to relatively small doses of the harmful substances that were contained in that particular sample of commercial meat extract became less and less, until finally with the restoration of normal metabolism the normal degree of susceptibility was reached.

Although partially thyroidless monkeys, and probably thyroidless dogs, and even men with disturbance of the activity of the thyroid gland, appear to be less capable of resisting the action of certain substances that are prone to arise in food-stuffs derived from the flesh of animals, further evidence is certainly desirable before it can be definitely asserted that the intoxication which follows the ingestion of those substances is in reality entirely identical with that variety of intoxication of which the cachexia consists. It is true that the experiments of Bogoslowsky and of Kemmerich, as well as the other experiments which have been related in Part I of this paper, point to the fact that an intoxication, with some of its symptoms usually very similar to some of those manifested during the cachexia, can be produced in normal rabbits, and even in monkeys, by means of large doses of meat extracts prepared from meat that has been kept for a variable length of time; but as my experiments in this chapter show, the symptoms of the cachexia will develop in the totally thyroidless dog or monkey when only cereals compose the diet both before and after the performance of the thyroidectomy. It is obvious, therefore, that although certain toxic substances which a meat diet may or may not contain, may still further poison the thyroidless animal, other factors probably arising within the organism, as Ewald has pointed out, must evidently be considered as playing a far more important part in directly producing the cachexia. As regards the experiments in the partly thyroidless monkey, it is evident that the most justifiable deduction from the results is that, owing to the disturbed general metabolic condition that temporarily results from the partial loss of the thyroid glands, the natural resistance of the animal to a certain toxin-containing food was greatly lessened, and in consequence the susceptibility of the monkey to such poisons became for the time being more prominent.

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* This hypertrophied residuum of the left lobe was removed after a period of 20 months. Although the animal possessed a well differentiated aortic body, 2.5 mm. in diameter, and was given fresh thyroid, iodothyrine (Beyer), and thyrocolloid prepared according to Hutchison's method, by mouth and by injection, after it had become myxœdematous, it did not survive longer than five weeks.

3. *Birds.* In Part I I found that I was not able to produce any signs of an intoxication in a normal cock by the administration of very large doses of various decomposed animal tissues, consequently it was determined to perform as complete a thyroidectomy as possible and observe if any of the symptoms of an intoxication or of the cachexia could be produced in this bird by a meat diet, for it is stated that an apparently total bilateral extirpation of the thyroid and accessory thyroid bodies does not produce the symptoms of the cachexia in chickens (Allara *) and pigeons (Ewald and Rockwell †).

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* Allara, *Lo Sperimentale*, 1885, p. 281.

† Ewald and Rockwell, Pflüger's *Archiv*, xlvii (1890), 160.

It is questionable, however, whether the preceding statement is to be accepted as final, for, as Horsley remarks, duration of life after the operation may play in thyroidless birds the important part it does in some other orders. Also the importance of bits of accessory and of embryonal thyroid tissue situated in unsuspected locations must not be forgotten, for, thanks to Gley, the importance of these apparently insignificant and easily overlooked bodies has been clearly demonstrated in rabbits and, thanks to Christiani, in rats.

After extirpating the thyroid and all of the adjacent accessory bodies that I could discover in a cock, it was fed for a number of weeks on meat, fresh, stale, and even slightly putrid, but no signs of an intoxication or of the cachexia could be detected. Wishing next to determine if the thyroidless bird had become susceptible to the substances contained in many of the dried thyroid preparations, it was given daily for three weeks large amounts (15 to 20 grm.) of the most toxic thyroid preparations that I had on hand. Lastly, I gave it by means of the tube the concentrated bouillon from 800 grammes of very stale sheep's thyroid, but none of these substances produced the least effect and the bird is still under observation.

Until, however, a careful dissection and a microscopical examination of possible residual accessory thyroid tissue is made, I refrain from drawing any conclusion from the above negative results.*

PART III. SOME OF THE PHYSIOLOGICAL ACTIONS OF CERTAIN ANIMAL EXTRACTIVES.

A. *The thyroid extractives and their influence upon the acute cachexia.*

1. *Historical introduction.*

In Part I it was stated that although the results of my experiences fully warrant the conclusion that the ingestion of even large amounts of the finely minced, absolutely fresh, raw thyroid gland for a considerable period of time is not followed by any signs of an intoxication, or by any symptoms that resemble in the least those of exophthalmic goitre, a certain amount of time might be necessary for the active principle or principles to develop in the freshly removed gland, exactly as is usually the case with an enzyme. In fact, a number of observers (White and Davies,[*] Greenfield,[†] Notkine,[‡] and others) hold that the active substance of the thyroid gland is probably an enzyme in character. In consequence of the preceding possibility it was found necessary to isolate the various components of the raw or of the dried complex thyroid material, and determine which of them is the active principle, and also if this active principle will induce an intoxication if given by the mouth in large doses to monkeys and other lower animals.

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* Since the above was written the bird has been killed, more than six months after the thyroidectomy. It must be admitted that the above experiment merely verifies the previous statement that normal cocks seem to be insusceptible to decomposed animal foods, for several small bits of tissue were found in the region of the right thyroid body that proved under the microscope to be accessory thyroid tissue. Probably these small bodies had hypertrophied from very minute embryonal accessory thyroid bodies, else I should not have failed to notice them at the time of the operation.

In conjunction with the experiments with the substances obtained from the thyroid gland, comparative experiments were also made with substances derived by similar methods from other animal glands and from muscle, and as some of the results that were obtained in the latter experiments have not been, as far as I know, hitherto described, a brief account of them is also included.

Evidently from the recent publications of Fraenkel, Baumann and Drechsel, a number of independent seekers after the active principle supposed to be contained in the thyroid gland have been at work at the same time as myself, and the preliminary results of one of these observers, Drechsel,[§] agree in many respects with my own independent observations, although the chemical process advocated by Drechsel differs somewhat from one of those employed by me.

S. Fraenkel [||] states in a preliminary report, read before the Society of Physicians of Vienna, November 22, 1895, that he prepared cold or warm extracts of known quantities of dried sheep's thyroid, and after precipitating the albumins by acetic acid, he found the filtrate active, reaching this conclusion from the fact that after drinking the neutralized filtrate daily for six days he lost 300 grm. per day during that period. By the process very analogous to that frequently used for the extraction of creatin—a process also used by me in general for extracting the fresh raw thyroid and thymus glands, muscles, etc.—he obtained, after removing the lead by sulphureted hydrogen, a syrup which was dissolved in alcohol and precipitated by means of ether or of acetone. This precipitate crystallized after cleaning. The crystals * were very hygroscopic, and permanent only in the "dried part." They were soluble in water and in alcohol, but insoluble in ether. By decomposition of the precipitates produced either by phospho-tungstic acid or by phospho-molybdic acid, with barium hydrate, a free base was obtained in the form of a markedly hygroscopic syrup. The results of the elementary analysis of the acetic salt led to the empirical formula $C_6H_{11}N_3O_5$. To the pure substance he applied the term "thyreoantitoxin." The physiological tests of this substance are also described by him, and will be referred to in detail later on by me.

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[*] White and Davies, *Brit. Med. Journ.*, 1892, ii, 966.

[†] Greenfield, ibid., 1893.

[‡] Notkine, *Sem. méd.*, 1895, p. 138.

[§] E. Drechsel, *Centralbl. f. Physiol.*, ix (1896), No. 24.

[||] S. Fraenkel, *Med. Record*, Jan. 11, 1896.

Baumann † was the first to discover that iodine, probably in combination, exists in the thyroid gland, and concluded from Roos' ‡ results in the reduction of goitres by the iodine compound that this compound, or "thyreo-iodine," is probably the active principle of the thyroid gland. The method of isolating the thyreo-iodine is not described in Baumann's article.§

Drechsel, after trying numerous undescribed methods, chose the following one which led to crystalline products:

The fresh pig's thyroids were minced, and repeatedly extracted with water at 35° C. The aqueous extract after acidifying was boiled, filtered and evaporated to a syrup, which was filtered after standing. The filtrate was precipitated with phospho-tungstic acid, and this precipitate, after being washed free from chlorine, was repeatedly boiled in water until no more would dissolve, the insoluble part being finally dissolved in an ammoniacal solution. Both solutions were then decomposed with baryta water, the ammoniacal being previously treated with CO_2 and then warmed until all ammonia had been driven off. The filtrates were then exactly neutralized with H_2SO_4. The residue of both solutions crystallized. According to Drechsel both of these crystalline products proved active when fed to thyroidectomized dogs, although not very markedly so. Fraenkel's base this author considers as identical with one of the two he describes, although Drechsel does not specifically state

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* S. Fraenkel (*Wiener med. Blätt.*, 1895, 13-15) considers these crystals as the acetic salt of the base thyreo-antitoxin.

† Baumann, *Zeitschr. f. physiol. Chem.*, xxi (1895), 319.

‡ Roos, ibid., xxi (1895), 19.

§ Baumann and Roos, ibid., xxi (1895), 481. See appendix for method, etc., p. 217.

that he has obtained the two crystalline substances in a state of absolute purity. The comparative action of these substances on the blood pressure is also not referred to in that preliminary communication.

As the above-mentioned observers point out, the only way that one can possibly determine which is the true active substance or substances of the thyroid is to try to isolate the various substances and try them, preferably first on the lower animals after removal of the thyroid gland, and later on myxœdematous men. The former I have done, but owing to lack of human myxœdematous material I have not had an opportunity to test their action in that disease. The various substances were fed in several experiments to totally thyroidless dogs, but as these animals very early in the cachexia refuse food of any description and later swallow fluids either with great difficulty or not at all, the results, even when the minced raw gland was given, were so discouraging that I concluded to inject the solutions of the various extractives from which the albumin and nucleo-proteids had been removed. Naturally, as a control to these experiments, equal or considerably larger amounts of the extractives were also injected into normal animals and the effects carefully noted; for, judging from the previous experiments of Chamberland and Roux,* who noted toxic symptoms to follow subcutaneous injections of an alcoholic extract of spleen, and of Charrin and Ruffer † with bouillon, I considered that very probably all animal tissues would yield extractives which, if given subcutaneously in very large doses, would prove to be poisonous to many of the lower animals. From my investigations on this subject, so far as I have pursued them, I have no reason to alter that opinion.

2. *Methods of extraction.*

Of the various processes used by me in extracting the thyroid, thymus, various other glands and muscles, the following three appeared to yield the best results. One of these processes, namely, that frequently employed for the extraction of creatin, is almost identical with that described by S. Fraenkel, but the active mixture that I have gotten from the fresh raw thyroid by that method does not appear entirely to correspond to the alcohol-soluble crystalline substance that he describes. Furthermore, the physiological action upon the blood pressure appears to differ markedly from that obtained by him with the thyreo-antitoxin. The third process, although yielding a considerable amount of a gummy, resinous extract, which apparently also contains that thyroid principle or principles (Drechsel) which are beneficial to thyroidless dogs, is open to the objection that probably a certain percentage of the active material is lost from the employment of alcohol in the method, for, according to my experience, the more nearly absolute the alcohol the smaller is the amount of the thyroid substance that remains in solution. Briefly, the three methods most frequently employed by me for the extraction of the thyroid were as follows:

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* Chamberland and Roux, *Annales de l'Institut Pasteur*, 1888.

† Charrin and Ruffer, *Soc. de Biologie*, Jan. 26, 1889.

(*a*) The absolutely fresh raw thyroids (sheep, ox, calf) after mincing were mixed with about two volumes of cold distilled water and stirred briskly for twenty minutes. The mixture being continually stirred, was extracted for about twenty minutes at a temperature of 38° C. and then boiled for ten minutes. This hot bouillon was filtered through a fine linen cloth and the solid residue thoroughly expressed by means of a powerful press. After the grease had been skimmed off, the cool filtrate was acidified with acetic acid, boiled for ten minutes, filtered through fine paper and the cooled filtrate refiltered through paper. After evaporating this filtrate to a syrup and filtering again, the latter, after the acetic acid had been driven off by heat, was dissolved in a saturated solution of ammonium sulphate, and additional crystals of this substance were added to insure complete saturation of the whole mixture, which after standing was filtered through paper. The filtrate was decomposed with baryta water, CO_2 passed through the filtrate and all ammonium carbonate driven off by gentle heat. After repeated filtrations and evaporations a final brownish, waxy, hygroscopic residue was yielded. As the result of comparative tests of the various precipitates and filtrates, it was concluded that this residual waxy mass contained some of that part of the thyroid material which considerably influenced both the severity of the symptoms and the course of the cachexia in totally thyroidless dogs.

(*b*) The well boiled acidified thyroid bouillon, prepared as in the preceding method, was precipitated by lead acetate, filtered and excess of lead removed by means of sulphureted hydrogen. After testing the filtrate for lead it was evaporated on a water bath and, when it had reached the consistency of a thick syrup, it was carefully refiltered while hot. The syrup was further evaporated, ultimately yielding a dark brown wax-like mass, similar in appearance to that which had resulted by method *a*.

(*c*) To the filtered acidified thyroid bouillon, after it had been evaporated at 100° C. to about one-fourth of its original volume and allowed to become cold, one-half volume of ether was added and the whole well shaken in a stoppered vessel for about twenty minutes. After standing, the supernatant ether was decanted and the aqueous remainder filtered, all ether being finally driven from the filtrate by means of gentle heat. Three volumes of 95 per cent. alcohol were then added to the filtrate and the whole allowed to stand for twenty-four hours, the ensuing precipitate being then carefully filtered off. The filtrate was finally evaporated at 100° C., being repeatedly filtered during this process until the whole had become as dry as possible. This residue was dissolved in distilled water by means of heat, and after filtering the filtrate was evaporated at 100° C. until the reaction of the resulting brown gummy wax had become neutral or faintly alkaline. This wax appeared to contain most of the active substance, although the alcoholic precipitate appeared to be slightly active also, due to the fact, as I found out by other methods, that the waxy mass is but extremely slightly soluble in strong alcohol.

Although it was evident that these waxy hygroscopic masses which appeared to be very nearly similar, though obtained by three different methods, assuredly contained some of the thyroid principle—for subcutaneous injections of a 2 per cent. aqueous solution of any of the three considerably prolonged the existence of totally thyroidless young dogs—it was probable that the waxy mass also contained a number of other extractives. Consequently a number of methods were employed to subdivide the waxy masses and obtain the desired substance in a purer form, possibly in a crystalline condition. An aqueous solution of this wax was then precipitated, either by phospho-molybdic acid or by phospho-tungstic acid, and after carefully washing the precipitates with a 10 per cent. solution of the respective acids they were boiled in distilled water, the solutions allowed to become cold, filtered, and the filtrates decomposed with baryta water, neutralized with sulphuric acid and evaporated to a very thick syrup, which, on being set aside, deposited crystals of various kinds (5 to 8 varieties). The portion of the precipitate that was insoluble in boiling distilled water was dissolved as in Drechsel's method, in water rendered slightly alkaline with ammonia; the ammonia removed by CO_2 and heating the filtrate until all ammonia had been driven off. This residual solution was finally decomposed with baryta, neutralized with H_2SO_4, evaporated on a water bath and set aside to crystallize. But few crystals appeared when phospho-molybdic acid was used, the chief residual yield appearing to be an oily base-like substance. When phospho-tungstic acid was used the proportion of the various crystals was larger. These ultimate mixtures, containing 5 to 8 varieties of crystals in their oily mother liquor, were injected into totally thyroidless dogs, and each found to be active and capable of prolonging the lives of totally thyroidless dogs equally as well as injections of the mixed waxy extractives, and even better, according to my experience, than injections of unboiled aqueous or glycerine extracts of fresh raw thyroid glands.

Various attempts were made to separate the oily mother liquid from the various crystals and obtain the various crystals and the mother liquor in a state of absolute purity, but these efforts have been up to the present unsuccessful, consequently I cannot positively assert whether it is the oily or the crystalline substances. that are active. From two of these ultimate extracts, one by the phospho-molybdic acid method, and the other by the phospho-tungstic acid method, I succeeded by using amyl alcohol in removing some of the mother liquid, leaving most of the crystals undissolved, but neither the activity of the oily mother liquor nor that of the remaining crystals appeared to have been diminished, as well as could be judged from a test upon two totally thyroidless dogs. Thus it cannot be inferred that only the oily substances are probably the active parts of these two ultimate extracts. Whether one of these oily substances is the same as the base isolated by Fraenkel I cannot say, for, though ether precipitates it from an aqueous alcoholic solution, anhydrous alcohol dissolves scarcely a trace of it, and none at all after this trace has been several times precipitated by anhydrous ether and the supernatant alcohol and ether decanted. Fraenkel's active base appears from his account to be so soluble in alcohol that the alcohol-ether method is employed in extracting it in a pure form from the crude syrupy mixture. Further, both of the substances that I get give rise to very marked, though transient, alteration of the blood pressure when they are injected, singly or combined, directly into a vein, but Fraenkel states that no fall of the blood pressure follows the intravascular injection of his base, although acceleration of the pulse occurs.

The two active though impure products that I get from the wax extracted from the fresh thyroid probably correspond to those described by Drechsel, although it must be admitted that I have not been able to assure myself that the activity of the two products depends upon the presence of two different chemical bodies, for, as has been intimated above, I have isolated neither principle in a chemically pure state. It is possible that the two active products that are yielded by the waxy mass which contains various thyroid extractives owe their activities to an imperfectly performed chemical separation at the point when the phospho-tungstic or phospho-molybdic precipitates were partially dissolved in boiling water. I inferred this possibility from the fact that after dissolving one of the crystallized products in dilute alcohol and precipitating with ether, recrystallizing and removing as much of the mother liquor as possible with amyl alcohol and recrystallizing the undissolved crystals, the majority of the latter changed in form and crystallized exactly in the stellate form assumed by the majority of crystals yielded by the other crystallized thyroid product. An ultimate analysis of these crystals isolated in a pure form would have settled the question, but this, however, has not been attempted with my present chemical appliances.

It is noticeable that Drechsel does not specifically state in his preliminary communication that he has isolated the two principles in an absolutely pure form, nor does he state which of the crystalline substances occurring in the active residues are the true active principles. Drechsel's method, however, is evidently a more direct and simpler way of obtaining these substances than any of those mentioned above, and I have lately endeavored to isolate by that method the two active thyroid bases of Drechsel in pure form. Up to the present my several attempts have met with no better success in the case of these partially crystalline residues, which the method appears to yield, than they previously did when I attempted in the summer of 1895 to do the same thing with the residues obtained by one or other of the three methods which I have described above. The investigations on this subject, nevertheless, are still in progress.

B. *The effects of the thyroid extractives on thyroidless dogs.*

In the preceding section it was stated that the daily subcutaneous injection of even comparatively small doses of a 2 per cent. solution of the gummy, waxy mixture of the thyroid extractives before being treated with phospho-molybdic or phospho-tungstic acid, etc., into totally thyroidless dogs appears to exert a most decided effect upon both the course of the cachexia and upon the severity of its symptoms, consequently it was concluded that the active palliative thyroid substance was present in these extractives. I do not intend to convey the idea that I succeeded in keeping the totally thyroidless young dogs alive by this means for any great period of time, for I have never been able to do this either by the injection of fresh aqueous thyroid extracts or by feeding large amounts of fresh thyroid glands to the dogs, because all of my totally thyroidless dogs died in spite of the injection of the extractives in from ten to eighteen days after the operation. When the fresh aqueous unboiled thyroid extracts were injected eight to twelve days proved to be the greatest length of time that the lives of the thyroidectomized young dogs could be prolonged, provided the dogs had no tiny aortic or other accessory thyroid bodies. When compared with the results by the feeding methods which have been described in Part II, these results are very striking, for not only was the duration of life after the thyroidectomy further increased, but the intensity of many of the symptoms of the cachexia was very much lessened.

Briefly, the effects upon the cachexia of the subcutaneous injection of the fresh *crude* aqueous thyroid extracts were somewhat analogous to those of Gley and were as follows:

If the injections of the thyroid extract were begun on the day of the operation in daily doses of from 2 to 10 ccm. (each ccm. = about 1 grm. of raw gland), the dose varying with the weight of the dog at the outset, all of the dogs at times had mild convulsions, constant fibrillary muscular twitching and unsteadiness of gait, while occasional attacks of tetany were also prominent. When signs of an approaching dyspnœic attack were observed the injection was repeated. As a result the dyspnœa generally disappeared in the course of forty-five minutes or one hour. From the third or the fourth day the emaciation appeared to become more rapidly progressive, and steadily continued until the death of the animal. By the fifth or sixth day all food was refused and milk had to be given by the stomach tube. Even the milk was usually vomited in spite of previous cocainization of the fauces. Finally death occurred about the tenth or twelfth day, unless the dogs happened to have a sufficiency of small aortic or other accessory bodies. In the latter case the symptoms progressed more slowly, the tremor and restlessness frequently disappearing for several days at a time. In one dog with a large accessory aortic body all the symptoms disappeared after the lapse of ten days. When no injections of the fresh aqueous thyroid extract were given to the totally thyroidless dogs until convulsions had appeared, the only noticeable effect was a temporary moderation of the fits, the tetany and dyspnœic respiration being also considerably lessened, but the betterment was merely transient. In spite of frequent repetitions of the injections the fits recurred after one or two hours and continued to occur at intervals until the animals died, usually five or six hours after the attack had begun.

When enough of a sterilized 4 or 6 per cent. aqueous neutral solution of the extracted waxy substance was daily injected into the thyroidless dogs before any fits or dyspnœa had developed, the effect upon the symptoms of the cachexia was clearly more pronounced than that from the crude aqueous extract of the raw gland, and the prolongation of life was greater, the dogs not dying until the fourteenth or eighteenth day. In these dogs also the pronounced emaciation steadily progressed from the second day after the thyroidectomy until the end, although artificial feeding with milk was tried.

When about 30 minims of the 4 per cent. solution was tried as a beginning daily dose for a dog weighing 5500 grm. the quantity was evidently too small, for on the morning of the fourth day the dog was found in a comatose condition, breathing stertorously and having numerous fits. 100 minims were injected and one hour afterward the dog was able to stand up, although considerable stiffness remained in the hind legs. Two hours after, as the respiration of the animal had become very rapid and panting, and the cramps more pronounced, 75 minims were injected. On the next morning only moderate fibrillary muscular twitching and a slight degree of restlessness were present, the conjunctivitis, coryza and tetany having completely disappeared. From this date until the day of the animal's death 100 to 150 minims were injected daily, but as the dog would eat nothing and could not be induced to retain food artificially introduced into the stomach, death finally occurred on the fourteenth day.

When the fits were allowed to begin before any injections of the extractions were given I succeeded in causing the symptoms to subside only in those dogs that were injected very shortly after the beginning of the fits. If the dogs had been allowed to remain in the rigid, comatose condition and have numerous fits, although very large doses appeared to slightly lessen their intensity and to diminish the rapidity of the respiration, the totally thyroidless dogs did not recover from the attack, but died in from four to eight hours after it had begun.

After separating the gummy mixture of extractives into the two partially crystallized residual portions by treating it with phospho-tungstic acid, phospho-molybdic acid, etc., and injecting the residual portions separately or in combination into various totally thyroidless dogs, the resultant average prolongation of the animals' lives was practically about the same as if an equivalent quantity of the 4 per cent. watery solution of the unseparated waxy mixture of extractives had been used. When comparatively large quantities of either one of these two partially crystallized residual substances were injected, each of which appeared to be composed of crystalline products mixed with more or less oily mother liquor, the palliative effects upon the cachexia apparently differed in no way from those produced by injections of both combined in smaller amounts. All of these dogs died about the fourteenth or sixteenth day in spite of the palliative substance that had been introduced under the skin.

As the results of a number of further experiments with the thyroid extractives derived either by Fraenkel's or by Drechsel's methods have been invariably about the same as the above—not even as good when the fresh uncooked watery extract of the raw gland has been given—I am firmly convinced that it is impossible to prolong for any great length of time the life of a young dog that has been deprived of absolutely all thyroid and accessory thyroid tissue, by feeding the raw gland to the dog, or by injecting the water-glycerine extracts of the fresh raw gland or the concentrated, highly purified extractives isolated from the raw gland —an extended conclusion which has apparently been already indicated by the well-known experiments of Vassale, Gley, Hofmeister, Hertzen, Schwartz, Godart, Edmunds and others, with the crude aqueous or glycerine thyroid extracts.

C. *The toxicity of the thyroid extractives.*

It is evident, as I have remarked, that some of the active palliative thyroid substance is assuredly contained in the gummy extracted mass, and as absolutely fresh raw thyroid gland has been used for the preparation of this waxy mass, it is also evident that this active substance does not exist in the fresh raw gland, for an interval of only about twenty minutes occurred between the slaughtering of the animals from which the material was taken and the beginning of the extraction. Furthermore, from the manner of its extraction it is certainly not an enzyme, for boiling does not appear to affect its activity in the least.

Having obtained the active substance in this more purified concentrated form, it was necessary to give large doses of it to susceptible animals before I could pronounce upon its toxicity. Finally, from the indications of my previous results with the dried or the stale thyroid preparations, it was desirable to make comparative tests with extractives derived from the preparations which had proved to be very toxic to rabbits and monkeys when administered by the mouth.

Briefly summed up, I find that after giving the mixed extractives from the fresh gland to rabbits by the mouth in doses of 4 grm. (= about 220 grm. fresh thyroid) per kilo of rabbit, and continuing the same for even five or six days, the rabbits do not appear to manifest any symptoms at all, nor does the temperature rise above the normal.

To monkeys also were given as much as 4 grm. per kilo of body weight, but with entirely negative results. With the extractives from an average dried commercial thyroid preparation the results were totally different and deserve to be more fully described.

The extraction of the commercial dried thyroid gland was made by the acetate of lead method, the same method being also used for the fresh raw control material, and 0.25 grm. of the residual gummy mass, which the dried thyroid also yields, was given to a rabbit by the mouth. At the end of fifteen minutes the respirations of the rabbit had become about 225 per minute and were very shallow; the heart-beat was so feeble and rapid that it could not be counted. Considerable weakness in the hind legs was also present. In twenty minutes it lay sprawled out on its belly, with marked exophthalmos, and occasionally a slight muscular twitch would occur in one or other of the legs. Pronounced horizontal nystagmus next developed and continued for about ten minutes. Towards the end the respiration became slower and shallower and finally ceased, about forty-five minutes after the introduction of the dose. It was evident, therefore, that the toxic properties of the dried material had not been wholly, or apparently even partially removed by the chemical processes employed to obtain the gummy mass of extractives, which in this case were contaminated with a considerable number of crystals of chloride of sodium. Having treated the gummy mass, liquefied by heat, several times with 90 per cent. alcohol, I found that the undissolved portion still preserved its activity as far as its palliative influence upon the cachexia was concerned; also that this active residue had apparently been deprived of those constituents which were toxic to monkeys and to rabbits. Thus large doses of this active residue could be given to these animals by the mouth without producing any of the symptoms that the dried powdered gland had produced.

With the portion dissolved by washing the mass several times with the 90 per cent. alcohol, precipitating with anhydrous ether and dissolving the cleaned precipitate in water, the resultant toxic effects were extremely marked. As an average illustration of the effects of small doses to rabbits, the photograph reproduced in Plate XIV is appended. This rabbit was given 0.1 grm. by the mouth and the photograph was taken about twenty-five minutes after this dose had been ingested. The characteristic exophthalmos is fairly well seen, as well as the sprawled out position due to paresis of all four legs. Shortly after this picture was taken a marked, though temporary, horizontal nystagmus developed. The number of heart beats increased from 192 to about 286 per minute;

the temperature from 103⅖° F. to 105⅖° F., and the respirations from 60 to 80. At the end of four hours the rabbit was beginning to recover and by the next morning the above symptoms had to all appearances entirely disappeared. The rabbit now weighed 105 grm. less than it did on the previous day, but this loss was regained in the course of several days. When larger doses (1 grm.) were given to rabbits by the mouth the above symptoms rapidly developed, but were of very short duration. All the animals died within from 30 to 45 minutes, and two of the four so treated exhibited shortly before death an occasional spasmodic twitch in one or other of the extremities.

As this toxic and evidently impure ether-precipitate had been extracted from the gummy mass by means of dilute alcohol, I naturally supposed that at least a small part of the apparently harmless but active thyroid principle had also been dissolved by the dilute alcohol, and this undoubtedly was the case, although the palliative effect of this mixture was much less pronounced than its toxic effect. By washing the precipitate, produced by adding ether to the alcoholic solution, several times with boiling absolute alcohol, decanting the supernatant alcohol and injecting an aqueous solution of the oily remainder, which was insoluble in the absolute alcohol, into a totally thyroidless dog, the course of the cachexia appeared to be modified equally as if the mixed active extractives from the fresh raw gland had been used. The decanted alcoholic portion, when evaporated and dissolved in water, did not seem to possess this power, for the thyroidless dogs that were injected with small quantities of the solution promptly died between 50 and 60 hours after the thyroidectomy. If large doses of this solution of toxic substances were injected into thyroidless dogs when they exhibited a considerable degree of tetany and pronounced prodromal signs of an attack of dyspnœa, the effect appeared to be that the symptoms of the cachexia became entirely altered and gradually gave place to those produced by the toxic substances. As some of the latter symptoms were to a certain extent the diametrical opposites of the former, the condition of tetany rapidly disappeared and all four legs soon became weaker, more flaccid, and finally completely paralyzed, so that the dogs lay sprawled out upon their bellies. Slight exophthalmos replaced the enophthalmos and, except for an occasional muscular twitch in the legs, no general convulsions, as far as I know, occurred. But as all these dogs were found dead on the morning following the afternoon on which the injections had been made, convulsions may have occurred during the night. Although it cannot be said that the lives of these dogs were prolonged by the toxin-containing mixture which the alcohol extracted from the waxy mass of extractives made from the dried thyroid material, the temporary and usually rapid effects following its injection in doses capable of producing the intoxication with its accompanying paralysis, etc., might, it is conceivable, lead one to believe that a large dose of the proper palliative thyroid substance had been injected, especially if the dogs were observed only during the transitional period between the subsidence of the tetany and the onset of the paralysis. Such a conclusion would certainly be erroneous, for, though the thyroidless dogs possibly got in this instance a very small amount of the proper palliative thyroid substance which the strong alcohol had dissolved, I have been able to produce identically the same effects by injecting thyroidless dogs with solutions of poisonous extractives obtained from various commercial meat extracts which also produced the above-described variety of intoxication in normal rabbits.

In the waxy mass of thyroid extractives prepared from the dried glands there appear to be at least two substances with entirely different physiological actions. These are:

(*a*) The principle or principles (Drechsel) that act beneficially upon the experimental acute cachexia thyreopriva.

(*b*) The principle or principles that are capable of producing an intoxication in certain animals when given by the mouth or by subcutaneous injection.

The first (*a*) is evidently the same as that which can be extracted from the fresh raw gland, and is apparently innocuous when given by the mouth to rabbits and to monkeys in extremely large doses. The second, or the toxic principles, does not prolong the lives of the totally thyroidless animals and, moreover, does not appear among the extractives when the perfectly fresh thyroid gland is employed. From their chemical behavior it cannot be said that they are enzymes. Nor can it be said that the intoxications that they produce are specific for the thyroid only, for substances capable of producing intoxications similar in every respect to those produced by the thyroid toxins appear to exist in the extractives from other stale animal tissues, muscles, spleen, thymus, etc. It is evident, therefore, that the thyroid substances which prove toxic by way of the stomach to normal rabbits, monkeys and probably also to men, arise during the post-mortem chemical alterations which the constituents of various animal tissues, and especially those of the thyroid gland, are more or less prone to undergo.

Are the mixed extractives of the fresh thyroid gland poisonous to animals when large doses are injected beneath the skin? Most certainly, according to my experience, but not more so, or even frequently not as much so, as some of the extractives derived by similar chemical methods from the fresh suprarenal capsules, the spleen, the liver, the muscles, the salivary or the lymphatic glands, the spinal cord and the blood (dog). But because all the above dead animal tissues will yield extractives that are poisonous when injected in rather large doses under the skin of an animal, one is not, however, justified in concluding forthwith that these poisonous extractives normally enter the blood in this form from the living tissues of the body. Consequently I do not think that the subcutaneous injection of the mixed extractives obtained from a supposedly internal secreting ductless gland by the above chemical methods, which very probably bring about extensive molecular changes in the original living constituents of the glandular tissue, truly indicates whether the supposed normal internal secretion, as it exists in the living body, can be toxic or not if it is produced in too great a quantity.

The only reliable method of testing whether or no the impure active thyroid principles are capable of producing an intoxication is to give large doses by the stomach, for both chemical and experimental evidence clearly demonstrate that their palliative activity on the cachexia is not lessened when they are absorbed from the alimentary canal.

Clinical and experimental tests have also clearly shown that when certain thyroid preparations are taken into the stomach by healthy or by diseased individuals they are capable of producing an intoxication with symptoms resembling in some respects those of Graves' disease. The digestive juices, therefore, destroy neither the active palliative thyroid principles nor the toxic principles. The raw fresh gland does not appear to contain the latter, but does contain the former, an apparently harmless substance even when ingested in enormous amounts; therefore, if the negative results of the tests by the stomach be considered valid, it is extremely probable that in exophthalmic goitre it is not the supposed increase of the normal active palliative thyroid principle which is responsible for the symptoms of that disease.

Are the toxins which one only finds in thyroid material that has been kept for a number of hours after the death of the animal, in reality identical with those toxic substances which the adherents of the hyperthyroidation theory of exophthalmic goitre claim the enlarged, or even the unenlarged thyroid gland is contributing to the blood? Judging from the extremely small amounts, frequently but 15 grains, of many of the dried thyroid preparations that will produce death in a strong, healthy monkey, I should say that the substances are not the same. The toxicity appears proportionately too great, for should a considerable quantity of such substances continuously enter the blood, I judge from my results in monkeys that rapid death would occur.

When but fifteen grains of a normal sheep's thyroid gland contain enough of these toxins to kill a monkey or a rabbit, it is legitimate to think that the enormously increased secretion of the supposedly over-active and often extremely enlarged thyroid in exophthalmic goitre would contain many times the quantity of toxins that are contained in fifteen grains of a normal gland. Did the internal secretion of the living goitre contain proportionate amounts of toxins identical with those of the dried normal gland, either a considerable degree of tolerance of rather large amounts of the toxins would probably follow, as the active secreting glandular elements of the thyroid gradually increased in amount, or if such tolerance were not established, very rapid death would occur, just as it does in an animal poisoned with the dried juice, or as it frequently does in man just after the partial excision of the struma. The result in the latter instance is due, according to the majority of surgeons (Kocher and others), to the inundation of the system by the thyroid juice unavoidably squeezed into the lymphatics during the manipulations of the operation, or to absorption of some of the poisonous juice which, according to Jaboulay, flows in enormous quantities from the gland after the performance of exothyropexie. When the enlarged living gland is continually adding such enormous amounts of a juice which ought, therefore, to contain an enormous amount of extremely toxic products, the degree of toxicity being judged by that which can be present in such small quantities of the dried normal sheep's thyroid, it is extremely probable that a certain degree of tolerance would occur, else the average case of exophthalmic goitre would not be liable to progress in the usual chronic manner. If the system of a patient with exophthalmic goitre become partially accustomed to the large continued inflow of the toxin-containing thyroid secretion, and were the poisons of that secretion identical with those that one usually finds in the poisonous thyroid preparations, one would hardly expect that the ingestion of the additional quantities of the supposedly similar toxins that are contained in one five-grain thyroid tablet per day for only one day would be followed by any very rapid and pronounced aggravation of the symptoms of the disease, especially as a considerable degree of tolerance of the thyroid toxins can be produced in some of the lower animals, and the same thing has been observed by clinicians to occur in man. Thus from a series of experiments on rabbits and monkeys I found that by very gradually increasing the daily dose of a poisonous thyroid preparation until relatively large amounts of the preparation could be ingested without producing any very pronounced symptoms, a fair degree of tolerance of the toxins that were contained in that variety of thyroid preparation could be produced. For example, a monkey that had been accustomed in this manner to 3 grm. doses of a preparation, took that quantity daily for five days and showed but very slight symptoms, whereas, when another monkey of the same species, that had previously received no thyroid, was given 20 grains of the same preparation, the monkey died in five hours.

Even a slight degree of tolerance of the poisonous thyroid preparations does not, however, appear to be present in exophthalmic goitre, a fact fully substantiated by the reports of Auld, Dreyfus-Brisac, Béclère, Ewald, Ingalls, Nasse, Stabel, Starr, and numerous others besides myself. According to my experience some of the symptoms of the several cases of exophthalmic goitre, to which I very cautiously prescribed small doses of commercial thyroid tablets that were subsequently found by experiment to contain a considerable proportion of toxic products, became apparently aggravated as early as the morning of the second day of the treatment, although but two 5-grain tablets had been taken in some instances. But the finely minced, absolutely fresh raw sheep's thyroid did not produce, as I have previously pointed out, this exacerbation of the symptoms; in fact, the patients with Graves' disease seemed to bear both the fresh gland and the extractives of the fresh gland very well.

Further, although the urine of individuals with Graves' disease has been demonstrated by Chevalier, Boinet and Silbert, Albu and others, to contain a larger percentage of toxins than that of normal individuals, none of those writers has demonstrated that the toxins excreted in the poisonous urine of those patients were identical chemically with those met with in the poisonous thyroid preparations.

I may furthermore add that the various poisonous thyroid preparations do not invariably produce symptoms that resemble those of exophthalmic goitre. Some of the symptoms may resemble those of the latter disease, but frequently marked differences are met with in the experimental intoxication, and furthermore I reiterate that toxic substances that produce identically the same symptoms as those resulting from the ingestion of the poisonous thyroid material may develop in other animal tissues. The latter poisons vary also in quantity and quality just as the thyroid poisons do.

From the above evidence it cannot be said that it is by any means conclusively indicated that the toxic substances which may or may not be found in certain thyroid preparations are identical with those that many observers suppose are being contributed to the blood by the enlarged thyroid in Graves' disease.

APPENDIX.

As practically the whole of this paper was already written before the publication of the second contribution of Baumann and Roos,[*] in which is described the method of preparing the principle "thyro-iodine," which they consider to be the active substance of the thyroid gland, my experience with that body must be related briefly in this appendix.

Baumann's method is as follows: The thyroid glands are boiled in a 10 per cent. solution of sulphuric acid until a nearly complete solution of the glands occurs. The active substance is for the most part contained in the fine, flocculent precipitate which also contains fat, fatty acids and other substances. The precipitate is repeatedly boiled in 90 per cent. alcohol with the result that the "thyro-iodine" gradually goes into solution in the alcohol. The alcoholic solution is evaporated and the residuum triturated with milk-sugar. After extracting the triturate with ether and benzin, it is dissolved in a cold dilute solution of caustic soda. On acidifying this solution the thyro-iodine is precipitated. Having redissolved it in caustic soda solution and reprecipitated it with HCl or with H_2SO_4, the coloring matters are separated and the thyro-iodine is obtained, after drying, in the form of a brownish powder that is insoluble in water but soluble in caustic alkalies. By this process 25-30 per cent. of the original iodine combination is lost. The greater portion of the thyro-iodine is united in the thyroid gland to two proteid bodies, "thyro-iodo-globulin" and "thyro-iodo-albumin," and only a small portion of the thyro-iodine is present as such in the thyroid gland.

Roos[†] has lately investigated the toxicity of thyro-iodine—a preparation that had been manufactured by F. Bayer & Co. according to Baumann's process—and in reference to Lanz's conclusions that the symptoms of the thyroid intoxication are due partly to the action of the thyroid material, and partly to the action of decomposition products, writes, "Eine Ansicht, die durch die Versuche mit dem Thyroiodin, wobei von irgend welchen Verunreinigungen durch Bacterien oder Zersetzungsproducte keine Rede sein kann, ihre Bestätigung erhalten hat." Incidentally, I must add in this connection that, judging merely from the odor of some of the bottles of thyro-iodine or rather "Iodo-thyrine" that were procured from that firm, one would be inclined to believe that a certain amount of decomposition had occurred in the original raw thyroid material, probably during the time consumed in collecting a large number of glands at the slaughter-house and forwarding them to the manufactory, where further time is consumed in carefully selecting the healthy glands. Be this as it may, however, Roos obtained (Exp. 2) from three doses (altogether equal to 18 grm. of raw gland) of this thyro-iodine preparation, headache, tremor, diarrhœa, palpitation and accelerated heart's action (108) with a temperature of 36.8° C. From larger doses (the equivalent of 18 grm. of raw gland given in two days) practically the same symptoms occurred, but the induced condition was never serious and the symptoms ceased after discontinuing the preparation. The experiences of Grawitz [*] and of Hennig [†] with this commercial thyro-iodine preparation also confirm those of Roos.

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[*] Baumann and Roos, *Zeitschr. f. physiolog. Chem.*, xxi (1896), 481.

[†] Roos, *Zeitschr. f. physiol. Chem.*, xxii (1896), 18.

On the other hand Ewald,[‡] discussing the effects of thyro-iodine on man, remarks as follows: "Vorübergehende Anwendung des Thyrojodins selbst bis zu 4 grm. pro die, hat keinen erheblichen Einfluss auf den Puls. Von Jodintoxication kann nicht die Rede sein, weil die Menge von Jod in der Drüse und ihren Präparaten sehr gering ist. Dagegen, werden Intoxicationen überhaupt beschrieben, wie Steigerung der Respirationsfrequenz, Kopf- und Gliederschmerzen, Speichelfluss, Urticaria, Herzklopfen, Zittern, u. s. w. Oft genug tritt aber trotz Anwendung grosser Quantitäten sicher wirksamer Präparate keine Reaction ein." According to Schuster [§] even the ten- or twenty-fold dose of thyro-iodine causes no disturbance of the pulse in man, nor does the intravenous injection of thyro-iodine cause disturbance of the blood pressure or of the pulse (animals?). Nevertheless, Baumann [||] states, "Schilddrüsenpräparate mit welchen man jene Vergiftungserscheinungen überhaupt nicht hervorrufen kann, werden überhaupt wenig wirksam sein." Further, Baumann calls attention to the more rapid action of thyro-iodine as compared to that of the raw gland or thyroid tablets, and explains the occurrence on the ground that the thyro-iodine must first be separated from the constituents of the thyroid material by the process of digestion—a statement which, in conjunction with the fact that thyro-iodine is insoluble in water and exists in very small amount as such in the raw gland, is, to say the least, very irreconcilable with the previous observations of others as regards the activity of crude aqueous extracts in myxœdema.

As the preceding evidence concerning the toxic nature of thyro-iodine derived from supposedly fresh thyroid material still leaves the subject in doubt, especially as all those observers who note the occurrence of toxic symptoms from the administration of thyro-iodine appear to have employed commercial thyro-iodine preparations which were obtained from the same firm, my own results with evidently active thyro-iodine preparations, prepared by myself and carefully tested for iodine, are appended.

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[*] Grawitz, *Münch. med. Wochenschr.*, 1896, No. 14.

[†] Hennig, ibid., 1896, No. 14.

[‡] Ewald, XIV. Congress f. inn. Med., Wiesbaden, April 11, 1896. *Wiener med. Wochenschr.*, 1896. No. 10, 376.

[§] Schuster, *Wiener med. Wochenschr.*, 1896, No. 10, 379.

[||] Baumann, *Münchener med. Wochenschr.*, 1896, No. 14.

After preparing a large amount of thyro-iodine according to Baumann's method and testing it for the presence of iodine, 2 grm. of the dried powder (unmixed with milk-sugar) were suspended in warm water, the acid solution was neutralized and given to a rabbit by means of the stomach tube. No immediate or remote effects on the heart-beat, the temperature or the eyes could be detected. No diminution in weight resulted.

A Java monkey was given 8 grm. of this preparation and two days later 10 grm. were given. As with the rabbit the result was negative.

To a patient with an enlarged thyroid gland daily doses of 1 grain of this thyro-iodine triturated with a little milk-sugar were given for a week. At the beginning of the treatment the circumference of the neck was 13½ inches; on the ninth day the neck measured 12¼ inches. No disagreeable symptoms appeared and, as this particular preparation was exhausted, the patient was put on 1 grm. doses of the commercial "iodo-thyrine" (1 grm. = 1 grm. of gland or about 3 milligrammes of purified thyro-iodine). After a week's administration of the latter preparation the neck measured but 12 inches. So far no disagreeable symptoms have occurred in this patient from doses of the above amount, nor has the patient diminished in weight. Possibly were larger doses to be given to this patient this preparation might give rise to toxic symptoms similar to those that Roos has observed with preparations from the same source.

Owing to lack of opportunity the thyro-iodine preparation made by myself was not administered to a patient with myxœdema. I am, therefore, unable to pronounce upon its activity in palliating that disease, but, judging from the diminution of the goitre which occurred during its administration, I am of the opinion that the preparation could be properly considered an active one. The toxic properties of commercial thyro-iodine have not been investigated by me in animals, but the experiments of Ewald indicate that the toxicity of the various lots probably varies like that of the various commercial thyroid powders and compressed tablets.

According to Baumann * the thyro-iodine is the only active thyroid

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* Baumann, *Münch. med. Wochenschr.*, 1896, No. 20.

principle, and from experiments on two dogs, he and Goldmann conclude that small doses of thyro-iodine are fully capable of completely replacing the thyroid function in animals (dogs) that have been deprived of that function through removal of the thyroid gland. Gottlieb's * results with thyro-iodine in thyroidless dogs do not, however, agree with those of Baumann and Goldmann, and my own results in *totally* thyroidless dogs with concentrated extracts obtained from thyroid glands by means of salt solution, which, according to Baumann and Roos, extracts all of the iodine combination from the minced gland, lead to the belief that the conclusion of Gottlieb that thyro-iodine is not capable of keeping the thyroidless dogs indefinitely alive is perfectly correct. Moreover, other experiments with purified thyro-iodine yield results that fully confirm the above conclusion of Gottlieb.

From the clinical results of Ewald and others with commercial thyro-iodine in the treatment of myxœdema, it is evident that the thyro-iodine prepared by the method of Baumann does contain something that influences the symptoms of that disease. Wishing to find out whether or no the iodine-containing product which Baumann's method yields would furnish further residual products if it was treated according to Drechsel's method of extracting the thyroid gland, four grammes of the powdered iodine-containing thyroid product were boiled in distilled water, the decoction being neutralized by a small quantity of caustic soda solution. After this solution had cooled it was filtered and the filtrate precipitated with phospho-tungstic acid. This precipitate was treated as Drechsel describes and ultimately yielded a small quantity of gummy residue that contained no iodine, but which after being dissolved in water appeared to possess physiological properties similar to those of the residue resulting from the ammoniacal solution of the phospho-tungstic thyroid precipitate in Drechsel's process. The chemical nature and composition of the gummy residue obtained from the iodine-containing product that was extracted from the thyroid gland according to Baumann's method have not been investigated by me except to test it for iodine, therefore I am unable to give further information as to its origin.

It is evident that the above-described facts concerning the production of toxic symptoms similar to those of exophthalmic goitre still harmonize with the general results discussed elsewhere in this paper. Also it is readily conceivable that should the least post-mortem decomposition have occurred in any of the thyroid glands before being put through the various stages of Baumann's process, some portion of the toxic substance or substances might still contaminate the thyro-iodine, so that in the hands of some observers one lot of thyro-iodine would give rise to certain disagreeable symptoms, while other observers using thyro-iodine prepared from a different lot of thyroid material would not observe the occurrence of those symptoms, even though very large doses were given. Even in a city of the size of New York, where large numbers of sheep and oxen are slaughtered daily, it is exceedingly difficult to gather together large amounts of absolutely fresh thyroids from animals that have been killed but a few minutes, and it is extremely likely that when very large quantities have to be collected for the preparation of thyroid products on a commercial scale, and twelve to twenty-four hours elapse between the death of the animals and the extraction of the glands, some decomposition has occurred in the material.*

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* Gottlieb, *Deutsch. med. Wochenschr.*, 1896, No. 15, 235.

D. *The effect of the thymus extractives upon the acute cachexia in thyroidless dogs.*

Concomitantly with the previously mentioned experiments with extractives derived from the thyroid gland, I have also carried on a series in which the thymus gland of the young ox served as the source of the extractives.

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* Hutchison (*Journ. of Physiol.*, xx, 1896; *Brit. Med. Journ.*, 1897, 194) states that the active part of the thyroid is found in the colloid substance which he finds is precipitated by acetic acid and redissolved by excess of acid. From chemical experiments performed since the publication of his papers, I find that after precipitating cold alkaline aqueous extracts of fresh sheep's thyroid with acetic acid, some of the substance that palliates the cachexia still remains in solution. When this solution is further treated with acetate of lead, etc., this substance finally appears as one of the probably many components of the waxy mass of extractives. The greater the quantity of this substance in the waxy mass, the more active the mass of extractives seems to be. The presence of this substance in the mass that I have termed "extractives" possibly accounts for the different results that I have obtained by injections of the so-called extractives.

In rabbits and monkeys I have not been able to produce toxic symptoms by administration by mouth of colloid from perfectly *fresh* sheep's thyroid, although large doses were given. The colloid was obtained in the manner Hutchison describes, but the *fresh* glands were extracted only for one hour. The toxicity of colloid after an 18 hour extraction of the *fresh* glands has not been tested, but from Hutchison's account of the rise of temperature, etc., after doses as small as .05 gm., its toxicity can evidently be considerable. (January, 1898.)

Only the perfectly fresh glands were used after being carefully examined for accessory thyroid bodies and were treated preferably by the acetate of lead method, which was also employed in the extraction of creatin and other constituents of muscle, and which Fraenkel * first recommended for the extraction of the substance that he calls "thyreoantitoxin." All of the methods that have been given for the extraction of the fresh thyroid apparently yield the active substances to which I refer in this section, but the acetate of lead process has always proved the least laborious and yielded considerable quantities of the waxy mass, consequently I have usually employed it.

Whether or no the process recommended by Drechsel for the extraction of his two thyroid principles will yield from the thymus substances that possess a physiological activity which is similar to that possessed by the products which I have obtained from the thymus by the acetate of lead method, I am not at present able to state positively. So far I have applied Drechsel's method to the thymus but twice. As in the case of the thyroid, the method yields with the thymus gland two brownish oily products, both of which contain numerous varieties of crystals. Many of these crystals are evidently identical with those that the thyroid gland yields by Drechsel's method, if one judges by their microscopical appearances only, but it must be admitted that I have met with no better success here in obtaining either these thymus crystals or the mother liquor in an absolutely pure state, than I did when the fresh raw thyroid gland was extracted according to Drechsel's method. One of these mixed crystalline products evidently possessed considerable activity, but the results with the other are not at all conclusive, for the five thyroidectomized dogs to which the substance was given proved at the autopsy to have moderately well developed accessory thyroid glands in the aortic region. Further experiments at present in progress will be necessary before I can conclude definitely whether both of the products that the method yields possess activity.

Precipitation of a solution of the brown gummy mass of thymus extractives that results from the acetate of lead method, by phospho-molybdic or by phospho-tungstic acid, boiling the precipitates first in water and then in an ammoniacal solution, decomposing with baryta, etc., appears to yield with the thymus also two active portions containing sundry varieties of crystals which deposit from the mother liquor. Whether the active principles of the two portions are really two chemically different bodies or not I am not prepared to say in this preliminary

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* Fraenkel, loc. cit.

notice of the phenomena that I have observed to follow the injection of these thymus extractives. If the active principles be two different chemical bodies, both are evidently contained in the gummy waxy mass which the thymus, like the thyroid, yields by the acetate of lead process. This waxy mass of mixed thymus extractives has, therefore, usually been employed, and the following remarks refer to its effects unless it is specifically stated to the contrary.

Before entering into the description of the actual experiments with the thymus extractives I deem it pertinent in this connection to state briefly that at the outset of the experiments a filtered thymus bouillon was employed. This of course was concentrated by boiling, filtered, and a large quantity injected into two thyroidless dogs that presented marked prodromal symptoms of an impending dyspnœic attack, considerable conjunctivitis and rhinitis, enophthalmos, frequent muscular twitchings, and considerable stiffness in the hind legs. In about one hour both animals appeared to be improved, for the symptoms of the impending attack subsided. Owing to the lateness of the hour no further observations of the animals were made until the following morning, when it was found that the thyroidless dog that had received the concentrated bouillon to the amount of 122 ccm. (= 244 grm. of fresh thymus) was lying sprawled out and apparently paralyzed in the hind legs, but the dog was still able to elevate itself upon the forelegs and drag the rear part of the body along for a short distance in a very unsteady manner. When the animal attempted to move, slight muscular twitching appeared in the forelegs, but when the dog was lying quietly no tremor could be detected. The conjunctivitis and rhinitis had almost subsided and the eyes no longer appeared sunken. The animal took its food with fair appetite and recovered the use of its rear legs by the afternoon. The following morning the dyspnœa returned and the dog had several convulsions, dying about 98 hours after the thyroid glands had been excised. A most careful post-mortem examination revealed no accessory bodies.

The second dog that received but 55 ccm. of the thymus bouillon was also apparently much benefited by the injection, for the signs of impending dyspnœa gradually subsided and on the following morning were entirely gone. On this and on the two succeeding days the existence of the cachexia was only manifested by occasional fibrillary twitchings in various muscles, progressive emaciation, periods of restlessness, moderate conjunctivitis and the manifestation of but little desire for the food that was placed before the animal. On the fifth day the typical fits occurred in the very emaciated animal, and death followed about midday. At the autopsy several small accessory bodies were found in the aortic and in the thyroid regions, consequently this experiment indicated in my opinion absolutely nothing.

Although no accessory bodies were found in the former dog, a definite conclusion regarding the cause of the apparent, though temporary, diminution of symptoms could not of course be made from such imperfect data. Nevertheless, the peculiar experience was considered worth a more extensive and careful investigation, and the following description of one of my experiments, it is hoped, will convey some idea of the effects which I have repeatedly observed to occur in totally thyroidless young dogs after the injection of a boiled 4 or 6 per cent. aqueous solution of the waxy mass of thymus extractives.

Two young, healthy dogs, respectively weighing 8200 grm. and 5750 grm., were chosen. The thyroid bodies of both were quickly removed (ether anæsthesia) with strict aseptic precautions. On the following morning the injections were begun, dog (*a*) receiving a small dose of the thymus extractives; dog (*b*) a similar amount of a 4 per cent. solution of thyroid extractive which had been extracted from fresh raw thyroid, also by the acetate of lead method.

The condensed notes of the experiment are as follows:

Dog (*a*). 1st day: 19 minims of a 4 per cent. thymus solution injected.

2nd day: 38 minims were given, as the animal was very restless and the usual tetany and conjunctivitis were beginning to appear.

3rd day: 87 minims given in three doses during the day, as the limbs of the dog were very stiff, and a mild attack of dyspnœa occurred. Weight 8005 grm.; pulse 160; temperature 105⅗° F. The animal still takes its food.

4th day: 90 minims injected; dog exhibits considerable tremor.

5th day: 9.45 A. M. 30 minims injected. Animal much brighter and is able to run about, although fibrillary twitchings still appear in the hind legs. Weight 8300 grm.; pulse 140; temperature 103°. Conjunctivitis subsided. The animal begins to take but little food. 1.30 P. M. Symptoms of an impending dyspnœic attack being present, 50 minims were injected and repeated at 2.30 P. M., after which the distressing symptoms quickly subsided.

6th to 9th day: 125 minims per day injected in three doses. Dog very weak and emaciated. Weight 7400 grm. Has refused all food for the past two days and has to be given milk by means of the stomach tube. Most of the milk was vomited ten or fifteen minutes after the withdrawal of the tube. Had a slight fit on the ninth morning, but recovered shortly after the injection of 50 minims of the thymus solution.

10th to 13th day: 75 minims per day injected. Weakness and emaciation steadily increasing and no food is retained in the stomach.

14th day: 50 minims given, but the dog died about noon. Weight of animal at death 5800 grammes.

Autopsy: The most careful search failed to reveal the presence of accessory thyroid tissue.

Dog (*b*). Was given 19 minims per day of a 4 per cent. solution of the thyroid extractives until the morning of the fourth day, on which date the dog was found at 9.15 A. M. having a series of very severe fits. After several doses, in all equaling 104 minims, the dog recovered and in the course of two hours was able to run about, although a moderate degree of stiffness still persisted in the hind legs. From the 4th to the 9th day, on which the animal died, 25 to 100 minims of the thyroid solution were injected per day, the amounts depending upon the condition of the animal. During this period several slight dyspnœic attacks occurred, but no more general convulsions. From the 4th day the dog refused food, and from this day the progressive emaciation became more prominent, 950 grammes being lost in all. At the autopsy a small accessory thyroid body about 2 mm. in diameter, which contained a small quantity of colloid, was found in the aortic region.

In this instance the animal injected with the thyroid extractives for comparison died before the animal injected with the thymus. Generally both animals survive for fifteen or eighteen days, but not longer unless they possess a small amount of accessory tissue.

Normal dogs injected with equal amounts of the above solutions apparently exhibit no effects.

The results of the foregoing and of other similar experiments lead me to believe, after comparing the effects of the thymus extractives prepared by the above method, that the concentrated thymus extractives prove just as efficient in palliating the cachexia in totally thyroidless dogs as the thyroid extractives.

I do not desire that the above statement should be interpreted as meaning that the active extractives from the thymus and the thyroid are chemically the same. This question has not at present been answered. It is possible that extracts from other cellular organs may be found to modify the acute cachexia as well as the extractives from the thyroid gland.

I am fully aware that the periods of survival of the thyroidless dogs that were injected with the thymus extractives or even with the thyroid extractives, to which the following remarks likewise apply, will seem entirely too short if my results are compared with those of other observers who claim to have kept thyroidless dogs alive for many weeks or even months by the mere administration of thyroid material by the mouth. I have, however, previously called attention to the fact that although I have repeatedly given enormous amounts of raw thyroid or of the extractives from the fresh thyroid to large numbers of thyroidless dogs, I have never been able to obtain such results except in dogs that were invariably proved at the autopsy to have several accessory thyroid bodies, microscopic or larger in size. Whenever the supposedly thyroidless young dogs continued to take food with their usual appetites, or when the profound disturbances of nutrition failed to appear fairly rapidly, or, if at the end of twenty days the thyroidectomized dogs that were being subjected to the thymus or to the thyroid treatment appeared to differ but little from normal dogs, I always concluded that a certain amount of living and functionally active accessory thyroid tissue was still present in the dogs. At the autopsy the animals were examined most carefully in all those regions in which Piana, Fuhr, Wölfler, Wagner, Carle and others have stated accessory thyroid bodies could occur. As a result of this examination it was found that my opinion was invariably correct, and consequently it was not allowable to conclude that the extractives or the diet of raw gland were responsible for the modified cachexia and long survival of the dogs in a practically normal condition. Even the activity of the small accessory bodies is not always sufficient to keep the dogs alive, and in spite of the aid derived from large amounts of thyroid material, some dogs will die as early as the tenth or fifteenth day. In other dogs the activity of the inaccessible aortic accessory bodies seems to be greater, and aided by the thymus extractives, the thyroid juice, etc., the lives of the dogs may be so prolonged that the remaining accessory bodies become moderately hypertrophied and sufficiently functionally active to maintain, with the assistance of the injected thyroid juice or extractives, the metabolism of the dogs in a fair state of equilibrium for a number of weeks or even months. Usually, however, the thyroid function seems to be but imperfectly assumed by the remaining accessory bodies, and in spite of the injections the profound disturbances of nutrition appear and ultimately bring about a fatal termination. If all thyroid tissue has been removed from the dog it is impossible, as I have remarked elsewhere, to prevent the comparatively early occurrence of this fatal termination, even though enormous doses of a thyroid preparation that has proved to be extremely efficient in myxœdema be given. Further, the unintentional oversight of the smaller and evidently active accessory bodies (Plates XV and XVI) which frequently are recognized only with extreme difficulty, especially when they are located in the mediastinum, fully explains, according to my experience at least, the discordant opinions existing at present among the various previous observers who have administered thyroid to young thyroidectomized dogs.

As is indicated in the preceding remarks on the possible behavior of thyroidectomized dogs owing to the presence of small, and often unsuspected accessory bodies, it is very essential to definitely determine their presence or their absence before a true comparison of the influence which the derivatives of the thymus gland appear to exert on the cachexia can be made with that resulting from the employment of the thyroid gland or its concentrated extractives.

Naturally the knowledge of the above-described effects on the cachexia of the concentrated thymus extractives is not of service in explaining the functions of the thymus gland, but it may furnish a partial explanation for the favorable results that Owen, Mikulicz, McKie, myself and others have observed to occur in patients with exophthalmic goitre when the thymus method of treatment was employed. I write " a partial explanation," for, besides the effects possibly directly due to the action of the extractives, the beneficial effects resulting from the daily ingestion of a large amount of very digestible and easily assimilable food, such as the sweetbread is, must also be taken into consideration. Granting, however, that the beneficial results that have been noticed in exophthalmic goitre are due to the extractives, the following question at once presents itself: " How can substances such as the thymus extractives which, as I clearly show, strikingly palliate the symptoms of the acute cachexia in dogs, possibly benefit the symptoms of a disease that are stated by the majority of observers to become generally more pronounced when the usual thyroid preparations, presumably also containing principles which palliate the acute cachexia, are ingested? " Or, in other words, if the thyroid extractives and the thymus extractives are equally capable of temporarily supporting an animal that has been deprived of all thyroid function, how can the administration of these similarly acting extractives give rise to beneficial effects in a diseased condition which the present consensus of opinion states to be the result of increased thyroid activity?

To attempt to properly elucidate these questions would entail necessarily a detailed discussion of the entire and still unsettled thyroid theory of Graves' disease. The pros and cons of this theory have been discussed so fully within recent years by numerous writers that it is not necessary to set them forth in detail in this paper.

In spite of all the evidence brought forward to support the theory that the symptoms of Graves' disease arise in consequence of exaggerated thyroid function, it is nevertheless an admitted fact that cases exist that present besides symptoms of exophthalmic goitre many of the characteristics of myxœdema. But it is reasonably conceivable from the facts which the observations of Reverdin, Schiff, Kocher, Fuhr, Horsley, von Eiselsberg, Poncet, Zesas, Ord and others have brought to light concerning the sequence of the various groups of symptoms which arise as the result of the total suppression of the thyroid function, or even from a partial interference of its function, that this class of patients ought to exhibit, before the myxœdematous tissue changes occur in the myxœdematous variety of Graves' disease, some of the symptoms at least that arise during the earlier stages of the cachexia that supervenes when the thyroid function is sufficiently diminished. The most prominent early symptoms, or symptoms of the *neurotic* stage (Horsley) as taught by experiments on monkeys, dogs and other lower animals, as well as by the sequence of events that has been observed after operative myxœdema (Reverdin) in men, consist of the following: profound general weakness, restlessness and periods of depression, fibrillar muscular tremor (rate of clonus 8-10 per second), local and general tetanoid spasms, emaciation and anæmia, increased lachrymal, nasal and salivary secretion, accelerated heart-beat, moderate or considerable elevation of temperature (Herzen, Horsley, Ughetti, Alonzo, Rogowitsch, Formánek and Haškovec), dyspnœic crises, ocular disturbances, such as enophthalmos * with narrowing of the palpebral fissure, muscular palsies, corneal and conjunctival congestion, etc., lowered blood pressure, and frequently dilated peripheral arterioles manifested by the congested condition of the various mucous membranes. Later, trophic disturbances of the hair, nails, etc., occur, and various motor palsies are also not infrequent.

When the myxœdematous tissue changes have fully developed it has been clearly demonstrated both in man and in the lower animals that a reversal of the majority of the above symptoms is the rule; hebetude, increased weight, diminished secretions, subnormal temperature, slowed heart-beat, etc., thus replacing their opposite of the early stages of the cachexia. The enophthalmos with narrowing of the palpebral fissure, however, generally persists.

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* In two of my meat-fed thyroidless dogs with accessory aortic thyroid bodies, in one bread-fed partially thyroidless dog, and in a dog in which all the vessels of both thyroid lobes had been ligated with catgut, a considerable prominence of the eyes developed on the morning after the thyroidectomy. The exophthalmic condition was so noticeable that, before I had an opportunity to direct attention to it, it was previously remarked by a laboratory attendant and others who had not been forewarned to be on the lookout for the occurrence. In the latter dog the exophthalmos lasted for about two weeks, but gradually became less noticeable owing to the marked puffiness that developed in the dog's face.

Thus a great many of the symptoms proper to the early stages of the cachexia are, as Mackenzie, Gley and others have specially pointed out, strikingly like those that are usually manifested by the average patient with exophthalmic goitre. Therefore, if one considers the following facts, namely, (*a*) the remarkable similarity of many of the common symptoms of exophthalmic goitre to those of the first stage of the cachexia thyroidectomica; (*b*) the well-known tendency of the struma to become indurated or otherwise degenerated, and the clinical fact that the partial removal of the supposedly overacting struma from individuals that manifested only the usual symptoms of exophthalmic goitre, unaccompanied by myxœdematous tissue changes, has been followed by the appearance of pronounced tetany, general or local swellings, myxœdematous in character, and occasionally by death preceded by all the usual symptoms of the acute cachexia, one is led to the conclusion that an insufficiency of at least one of the various possible functions of the thyroid gland may be present in exophthalmic goitre without myxœdematous tissue changes being evident.

Thus, from our knowledge of the effects of the fresh thymus and thyroid extractives on the symptoms that result from an insufficiency of thyroid activity, it is but reasonable to expect that, if a partial "hypothyroidation" (Gley) is directly or indirectly responsible in many cases of Graves' disease, an exhibition of these extractives by mouth would probably be followed by more or less amelioration of many of those symptoms. My previously described observations with the fresh thymus and thyroid glands confirm this, but my observations with the dried thyroid preparations conform with the usual experiences of others, that the exhibition of the average commercial thyroid preparations, powders, concentrated extracts, etc., to patients who have Graves' disease but no evidence of myxœdematous changes, generally results in making the patients decidedly worse, an occurrence that is due, in my opinion, to the action of the toxic alteration products which further disturb the already disordered metabolism.

Typical myxœdematous patients are also well known to be very susceptible to the poisonous preparations, many well-known English, German, French and American observers advising extreme caution in the thyroid treatment of myxœdema, and in fact, as Ewald has pointed out, symptoms of intoxication may develop during the treatment of myxœdema without the appearance of any curative or beneficial effects. Sonnenburg's* experience which was related in the historical retrospect (Part I) clearly shows, however, that in operative myxœdema enormous quantities of thyroid may be taken without development of any toxic symptoms whatever, the patient even improving and gaining in weight from the very commencement of the treatment.

These observations, coupled with various facts described in connection with the symptoms that occur in rabbits and monkeys after the administration by mouth of the concentrated toxins extracted from stale or dried thyroid, clearly indicate that the prominent katabolic effects which rapidly occur in myxœdema, obesity, etc., in consequence of the ingestion of the average dried thyroid preparations, are probably almost entirely due to the action of the contaminating and varying cadaveric poisonous derivatives.

Further, one is almost led to believe that, although an increased susceptibility to these toxins exists in myxœdema, if very small amounts of these powerful stimulators of katabolism are given in combination with those thyroid substances which are capable of modifying the early symptoms of the acute cachexia, the myxœdematous individual may be somewhat benefited by the reduction in weight from the decided destructive metamorphosis of the myxœdematous tissues and the increased excretion, which the toxic varieties of the thyroid preparations have been experimentally shown to produce. I am not, however, in a position to substantiate the above by clinical proof, for, as has been already stated, the palliative and the toxic activities of the thyroid preparations in general use, at least in the United States, are so variable and the clinical reports describing the various effects that have followed their ingestion in large and in small doses are often so conflicting regarding the occurrence of early and pronounced destructive metamorphosis and consequent loss in weight in myxœdematous individuals who are being treated with the different thyroid preparations, that it is impossible to gain a more extensive information from those clinical reports than the following:

(1) The majority of myxœdema cases are improved by the various preparations, the improvement being accompanied by a considerable loss in weight and other trophic manifestations.

(2) Considerable improvement occurs, but even a gain in weight accompanies the improvement.

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* This observer evidently employed thyroid material in which no appreciable post-mortem alteration had occurred.

(3) Loss in weight accompanied by the appearance of very distressing symptoms follows, but no evident improvement in the various nervous and other symptoms of the myxœdema.

(4) Neither loss in weight nor improvement in any of the symptoms results until a number of different thyroid preparations have been tried.

These facts are extremely contradictory, but the above-mentioned possible dual activity of the average thyroid preparations will probably be found to be mainly responsible for the irregularities described by clinicians in connection with the treatment of myxœdema by the thyroid method.

As the results under the fourth heading indicate, some of the preparations contain scarcely any of the active extractives, although an examination of a large number of samples of various thyroid preparations, some of which proved absolutely inactive in palliating the acute cachexia in totally thyroidless dogs, showed that iodine, and presumably the thyro-iodine of Baumann and Roos, was present in considerable amounts in all of those preparations of the crude powdered gland. This substance may, as Roos states, be the substance which causes the reduction in the goitre, but reduction in the goitre will also follow the ingestion of the thymus gland (Owen, Mikulicz and myself), and the thymus contains, according to Baumann,* no iodine, a fact that has been repeatedly verified by myself. Other organic bodies besides thyro-iodine consequently possess the property of causing the struma to diminish. In fact, the recent experiments of Gottlieb † with the concentrated thyro-iodine prepared according to Baumann's method are confirmed by my own experiences with the inactive or, rather, non-palliative iodine-containing thyroid preparations. The conclusion reached by this writer, that the thyro-iodine is not capable of keeping thyroidless dogs alive, is in my opinion perfectly correct, and from his experiments I judge that chemically pure thyro-iodine does not influence in the least ‡ the cachexia in *totally thyroidless* dogs.

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* Baumann, loc. cit.

Baumann (*Münch. med. Woch.*, 1896, No. 14) states, since the above was written, that by using large amounts of calf thymus he was able to detect the presence of iodine in it, although the quantity of iodine was very small. I have repeatedly tested the thymus for iodine, but it did not appear to be present in any of the thymus glands that were tested. I have, however, found traces of iodine twice in the salivary glands of the ox, and once in the parotid gland of the dog. Possibly accessory thyroid bodies were present in the thymus tissue examined by Baumann.

† *Deutsche med. Wochenschr.*, Apr. 9, 1896.

‡ Experiments performed by me since this paper was sent to the editor show that some lots of commercial iodo-thyrine (Bayer) do moderately

Is the presence of all the principles which Fraenkel, Drechsel and Baumann describe necessary in a thyroid preparation in order that the preparation may produce all the good effects that one observes in myxœdema? This remains to be seen, for further very carefully conducted observations are certainly necessary to determine this point. And in conclusion I add that only absolutely pure principles should be employed for such observations.

E. *The intravascular injection of the extractives.*

1. *Intravascular injection of boiled thyroid extracts.*

While some of my subcutaneous injection experiments were in progress, Oliver and Schäfer * having investigated the blood pressure effects of intravascular injections of decoctions of various glands, published the fact that the intravascular injection of the thyroid extract gives rise to an almost immediate but transient diminution of the blood pressure by increasing the calibre of the arteries, although the beats of the heart remain at about the same rate and of the same strength. Schäfer † states, "Before this effect upon the blood pressure was discovered by us it had been shown by my co-worker, Dr. George Oliver, that the exhibition of thyroid juice and other preparations of thyroid in the human subject has a tendency to increase the calibre of the radial artery." Haškovec ‡ also noted the fall of the blood pressure and, besides, an acceleration of the pulse. Fraenkel tested the effects of intravascular injection of his thyreo-antitoxin and found that this produced an acceleration of the pulse but no fall of blood pressure. Nor does a cold aqueous extract of dried sheep's thyroid produce, according to this observer, a fall of blood pressure, although an increase in the number of heart-beats does occur. Fraenkel states that he does not understand this difference and, therefore, gives no explanation.

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influence the cachexia in totally thyroidless dogs and monkeys when the dose is rather large; other lots do not appear to affect the course of the cachexia at all, although the preparations contain iodine. When solutions of these active iodo-thyrine preparations were treated according to Drechsel's method they yielded small amounts of two brownish gummy substances, neither of which contained iodine. Injections of a mixture of these gummy substances into totally thyroidless dogs palliated the cachexia somewhat, but not as much as boiled aqueous extracts of fresh raw thyroid usually do.

* *Journal of Physiol.*, xvi, 1894; xvii, 1895, and xviii, 1895.

† Internal Secretion, *Brit. Med. Journal*, 1895, 341.

‡ Haškovec, quoted by Fraenkel, loc. cit.

In order to compare the blood pressure effects produced, according to Schäfer and Oliver, by aqueous decoctions of fresh raw or of dried thyroid material with those produced by the thyroid extractives which I had found capable of modifying the acute cachexia thyroidectomica in dogs, I repeated Schäfer and Oliver's experiments, and also performed a number in which the various thyroid and thymus extractives were employed. My results with the decoctions prepared from absolutely fresh sheep's thyroid and from calf thymus practically agree with those of Schäfer and Oliver; the injections produced, as these observers have pointed out, a transient fall in the blood pressure, although the strength of the heart-beats and the rate remained almost the same (Plate XVII, Fig. 1). I state " almost," for usually the rate diminished by three or four beats per minute, and frequently, if a very large amount of the concentrated neutralized decoction was injected, a diminution of eight or ten beats occurred. This slight diminution in rate, like the fall of the blood pressure, was but temporary, and as the blood pressure gradually returned to its original height, the number of the heart-beats became the same as before the injection. Although the above effects were striking when fresh decoctions of the fresh raw material were injected into the external jugular of etherized dogs,—especially when both vagi had been cut,—I found that if the decoctions were kept and allowed to become more or less putrefied, their effect upon the blood pressure became markedly less and less and finally disappeared.

In several different anæsthetized and morphinized dogs a number of reboiled and filtered thyroid and thymus decoctions in various stages of decomposition were injected, and though the majority of them produced scarcely any diminution in the blood pressure, several of the stalest solutions caused a transient acceleration of the heart-beat. In no instance, however, did the acceleration amount to more than eight beats per minute when 2 ccm. (= 2 grm. of raw gland) of the stale solution were injected.

When the dried commercial thyroid preparations were employed as the source of the decoctions—of course allowing the pulverized gland to macerate for a number of hours before boiling the mass—my results were not nearly so striking or so uniform as those produced by smaller doses of the decoction of fresh raw thyroid which had not been allowed to macerate more than a few minutes before boiling.

To sum up the cardiac and blood pressure effects of these decoctions of stale and dried material:

(1) Some produced a moderate fall of blood pressure, the number of heat-beats remaining unaltered or occasionally diminishing three or four beats per minute. The fall of the blood pressure was always more prominent after both vagi had been divided.

(2) Some produced apparently no effect at all.

(3) Some caused no fall of blood pressure, but the frequency of the heart-beat temporarily increased six to ten beats per minute when the vagi were intact.

(4) A decoction from one preparation invariably gave a faint increase in the blood pressure in a morphinized dog with both vagi cut.

Thus, in (1) it will be seen that the effects practically agree with those that, according to Schäfer and Oliver, the decoctions from the dried preparations produce, while in (3) the effects correspond with those that Fraenkel states are produced by the "thyreo-antitoxin." The results in (2) depended upon the probable absence of or, as I found subsequently after extracting large quantities of the dried preparations, upon a considerable decrease in the relative quantity of substances that produced the fall of blood pressure. In (4) it was clear that considerable alteration of the constituents of the preparation had occurred, a fact that was further proved by the failure of injections of the decoction to modify in the least the symptoms of the acute cachexia in totally thyroidless dogs.

2. *Intravascular injection of the thyroid and other extractives.*

Extending the above blood pressure tests to the ultimate thyroid and thymus extractives, the following extractives isolated by methods previously described in detail (page 203 et seq.), were tested and found invariably to produce a very decided fall of the blood pressure when they were injected directly into the external jugular vein or into the portal circulation. Briefly, these substances were:

(*a*) Solutions of the brownish, waxy, impure mixed extractives, obtained from perfectly fresh thyroid and thymus glands, by the three methods which we have described.

(*b*) The brownish waxy mass obtained from the waxy mass by treatment with 90 per cent. alcohol and repeatedly precipitating the alcoholic solution by anhydrous ether. (That from the thyroid should correspond to Fraenkel's thyreo-antitoxin.)

(*c*) The two crystalline mixtures obtained by decomposing the waxy thyroid mass with phospho-molybdic acid, etc.

(*d*) The two crystalline mixtures obtained by decomposing the waxy thyroid mass with phospho-tungstic acid.

(*e* and *f*) The crystalline mixtures from the thymus as in *c* and *d*.

(*g* and *h*) The two crystalline mixtures from the fresh raw thyroid by Drechsel's process.

(*i* and *j*) The two crystalline mixtures from the fresh raw thymus by Drechsel's thyroid process.

Intravascular injections of each of the above extractives produced effects practically similar to those produced by the decoctions of the fresh raw material (Plate XVII, Figs. 1 and 2, and Plate XVIII, Fig. 3). When neutral 5 or 10 per cent. solutions were employed the fall of the blood pressure was very pronounced, and frequently the number of heat-beats very perceptibly, though temporarily, diminished.

When the dried commercial thyroid preparations were used as the source of the extractives, it was found that generally the extractives from them affected the blood pressure in just the same way as the extractives from the fresh thyroid, but relatively small amounts of the extractives were yielded by most of the dried preparations, a circumstance which probably accounts for the results in (2) with the more dilute decoctions of those dried preparations.

Two especially foul-smelling dried preparations were extracted by the acetate of lead method, and the resultant waxy mass of extractives treated by alcohol, ether, etc., the cleaned ether precipitate dissolved in normal saline solution and injected. No fall of blood pressure followed, but a slight acceleration of the heart-beat (about eight beats per minute) was produced several times when 1.5 ccm. of a 5 per cent.

solution were injected at one dose. This acceleration, however, was often variable and frequently did not occur when the above quantity or even a larger quantity (2.5 ccm.) was given.

Comparing these effects with those produced by the various extractives obtained from the absolutely fresh raw thyroid, it was evident that the blood-pressure-reducing substances had undergone considerable alteration in many of the dried thyroid preparations. Why I cannot say, for I do not know exactly the process by which the dried preparations were prepared, nor do I know how long a time was consumed in the slaughter-houses in gathering the raw thyroid glands together for the various manufacturers of the desiccated preparations.

I have stated that if the extractives (*a-h*) were injected into one of the tributary veins of the portal circulation, a diminution of the blood pressure resulted, but, as might be expected, the abrupt fall that usually occurred when the injection was made directly into the external jugular vein was not produced. The total fall, however, was about the same as if an equal dose had been injected into the jugular vein.

Wishing to see if the extractives would affect the blood pressure after being absorbed through the stomach and the intestines, I injected into the stomachs of etherized fasting dogs very large quantities of the extractives *a*, *c*, *d*, *e* and *f* mixed, a mixture of *g* and *h*, and also one of *i* and *j*, but without producing the least diminution in the blood pressure.

The method pursued was as follows: After previously inserting a cannula into the carotid artery and determining the blood pressure, 15 grammes of *a* were injected into the empty stomach of a dog weighing 4300 grms., and a continuous tracing was taken during a period of two hours and twenty-five minutes, and except for slight irregular changes that one usually sees occur with prolonged anæsthesia, no alteration of the blood pressure could be detected. Had the same amount been injected directly into the external jugular vein almost instantaneous death would probably have occurred, for I have seen it occur when even 5 grms. of *a* from the thyroid or from the thymus were injected in this manner. Such enormous quantities appear to cause very profound chemical alterations of the blood.

Extractives from the aqueous decoctions of certain other glandular organs, for example the submaxillary glands of the ox, also produce a fall of the blood pressure, and so do the extractives yielded by muscle, and also those from commercial meat extracts. The effects of injecting one derived from an ordinary sample of Liebig's extract by the acetate of lead method are shown in Plate XVIII, Fig. 4, and no further comment is necessary except the remark that a transient increase of eight to ten heart-beats was usually produced by these injections.

From the above experiments it is evident that Fraenkel's thyreo-antitoxin, which, he states, does not lower the blood pressure, and the active palliative substance which I obtain from the perfectly fresh raw thyroid gland by the process that he recommends, possess entirely different properties in respect to their influence on the blood pressure and on the rate of the heart. The cause of this difference is at present inexplicable, unless it be that the dried thyroid material which he employed as the source from which he extracted his thyreo-antitoxin had undergone extensive alteration during its manufacture. The blood pressure effects that I have got with the thyroid extractives by Fraenkel's method, that is to say, with the extractives of those preparations that had undoubtedly modified the acute cachexia in totally thyroidless dogs, and with the two substances obtained according to Drechsel's methods, practically accord with those that Schäfer and Oliver noticed with the aqueous decoction of the raw glands.

But, though the transient fall of the blood pressure and tendency to slow heart-beats are produced by the intravascular injections of thyroid decoctions and extractives, this fact does not conclusively prove that the hypothetical internal secretion of the thyroid gland in the living body is necessarily continually affecting the blood pressure in this manner. On the contrary, injections of large amounts (30-50 ccm.) of the defibrinated blood from the inferior thyroid vein removed while the gland was being massaged, did not produce during any of my experiments the above effects; a fact which would of course only tend to show that none or, if any, an extremely small quantity of the blood-pressure-reducing thyroid substance was present in the blood after passing through the gland.

Nor can one assert from the results of the preceding experiments with the thyroid derivatives that the transient blood-pressure effects that are produced are specific for the secretion of the living thyroid gland, for derivatives that produce similar effects on the blood pressure may be readily obtained from the salivary glands (ox), from the muscles, etc. The latter certainly have no internal secretion, in the proper sense of the term, and with respect to the muscle extractives that lower the blood pressure the only definite fact my experiments indicate is that the proteid-free muscle extracts contain certain chemical substances that possess the properties of temporarily reducing the blood pressure when they are directly introduced in minute quantities into the circulating blood. They certainly do not indicate that these substances normally enter the blood or the lymphatics from the muscles in this particular chemical state.

Nor are the blood pressure and cardiac effects which the complex juice of the minced raw thyroid temporarily produces, when it is injected directly into the circulation, definitely apparent in exophthalmic goitre, in which disease too much internal thyroid secretion is claimed by many as being the chief pathogenic factor. Thus, in exophthalmic goitre, acceleration of the heart-beat is, according to all observers, usually the first of the cardinal symptoms to appear. Regarding the state of the blood pressure clinicians differ greatly, and from the fact that so many diametrically opposite opinions have been expressed, it is evident that the state of the blood pressure is not always the same in different patients with exophthalmic goitre. For instance, François-Franck and Marie have found the state of the blood pressure in Graves' disease to be normal.* Sachs † states that "it is owing to the increased tension that hemorrhages are frequent from the nose." Starr ‡ writes, "The condition of the heart and arteries in myxœdema is exactly the opposite of the condition found in exophthalmic goitre. In all the cases of myxœdema a slow pulse is present. The pulse is also small and of high tension in myxœdema. In sharp contrast to this condition are the rapid, full, large pulse and throbbing arteries constantly complained of in exophthalmic goitre." *

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* François-Franck and Marie, quoted by Chamberlain. Maladie de Basedow, Paris, 1894.

† B. Sachs, Nervous Diseases of Children. New York, 1895, p. 199.

‡ M. A. Starr, *Med. News*, April 18, 1896.

On the other hand the removal of the thyroid gland in dogs and monkeys is followed by a state of subnormal blood pressure, the very condition that the injections of thyroid decoction temporarily produce. In both varieties of animals the pulse-rate is accelerated also in the early stages of the cachexia, but as the myxœdematous stage supervenes (monkeys, rarely dogs), the number of heart-beats becomes reduced and falls usually below the normal.

It is evident, therefore, that many complicating and, at present, inexplicable factors are probably concerned in the vascular manifestations that arise in consequence of disturbed thyroid function. All that one can say from experiments of the foregoing character is, that the connection between the effects observed after the intravascular injections of aqueous extracts, etc., of ground thyroid tissue, and the various cardiac and vascular manifestations observable in conditions in which the supposed internal thyroid secretion is presumably disturbed, is entirely too indefinite in the present state of our knowledge of the presumed internal secretion to warrant the inference that the internal secretion of the living thyroid gland has for one of its functions the production of cardiac and vascular effects identical with those produced by the direct intravascular injection of the extracts, decoctions and various chemical derivatives of the thyroid tissues.

VI. CONCLUSIONS.

From the results of the various experiments already detailed I feel justified in drawing the following conclusions:

(1) Absolutely fresh thyroid gland is not poisonous, in the usual sense of the term, when absorbed through the alimentary canal.

(2) The symptoms of induced thyroidism are manifestations of an intoxication resulting from the ingestion of decomposed thyroid mater-

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* The blood pressure of six females with well defined exophthalmic goitre has lately been investigated by me with the sphygmodynamometer of Oliver. 130 to 150 grammes were necessary to just occlude the radial arteries. The blood pressure in these patients was thus normal, 120 to 160 grammes being the normal limit according to Oliver.

ial, a conclusion that agrees in part with the previously related observations of Lanz.

(3) The so-called experimental thyroidism is not specific for the thyroid only, for the ingestion of many substances derived from animal tissues other than the thyroid gland may produce an intoxication strikingly similar in every respect to that of experimental thyroidism.

(4) Most, if not all, animal tissues yield substances which, if injected in large quantities directly into the circulation or beneath the skin, will produce an intoxication often very similar to that produced by injections of various substances derived from the fresh thyroid tissue.

(5) The effects resulting from the intravascular or subcutaneous injections of aqueous extracts, decoctions and the concentrated extractives of the thyroid tissue, of the thymus, of muscle, etc., are by no means necessarily indicative of the function and the action of the hypothetical internal secretions of the same tissues during life.

(6) The utilization of the fact that ingestion of decomposed thyroid material produces on certain occasions an intoxication with certain symptoms similar to some of those of Graves' disease is not justifiable for the furtherance of the theory that the symptoms of exophthalmic goitre result from an over-production of the thyroid secretion.

(7) Our results lead us to conclude with Drechsel that the fresh thyroid tissue yields at least probably two substances that are capable of palliating the symptoms of the acute cachexia in totally thyroidless dogs.

(8) The thymus tissue also yields one and probably two substances that are as equally capable as the thyroid extractives of palliating the acute cachexia in totally thyroidless dogs.

(9) Neither of the above substances is an enzyme, nor does either contain iodine.

(10) Neither the feeding of minced raw thyroid glands, nor the injection of aqueous thyroid extracts, decoctions, and concentrated solutions of the extracted palliative thyroid principles is capable of keeping *totally thyroidless* young dogs alive longer than a few weeks (possibly three weeks). Still less capable are the thyroid preparations containing decomposition products.

(11) The presence of one, or usually several, small accessory thyroid bodies, which gradually hypertrophy and wholly or partially assume the functions of the excised thyroid lobes, accounts for the occasionally long survival of thyroidectomized, thyroid-fed, young dogs.

(12) Totally thyroidless young dogs are so quickly overwhelmed by the cachexia, and the intervals between the thyroidectomy and the onset of the severe dyspnœic attacks and subsequent deaths differ so slightly, no matter which of the usual varieties of fresh food are employed, that *kinds* of fresh food cannot be unquestionably affirmed to influence the onset of the cachexia in any especially definite manner. Animal foods, in which constituents poisonous to rabbits have developed, probably slightly hasten the onset of the severer symptoms, and the vaunted remarkably modifying influence of a diet of ordinary milk, such as Breisacher observed, does not exist in the case of the totally thyroidless dog.

(13) Monkeys whose general metabolism is disturbed in consequence of the removal of a greater portion of the thyroid gland, evidently become more susceptible to those constituents of meat that are poisonous to rabbits, and sufficient clinical evidence exists for concluding that probably a like susceptibility to animal foods containing such constituents also exists in men when the function of the thyroid gland is sufficiently disturbed.

(14) And, finally, as regards the thyroid factor in the pathology of exophthalmic goitre, I agree with Gley that the majority of the symptoms in many patients with that disease can apparently, from an experimental standpoint, be as plausibly explained by the hypothesis of partially *deficient* thyroid activity as by the hypothesis of augmentation of thyroid function.

DESCRIPTION OF PLATES XIV-XVIII.

Plate XIV.

Photograph of a rabbit with acute thyroidism after the ingestion of 0.1 gramme of a poisonous mixture of substances extracted from a dried commercial thyroid preparation. The acetate of lead method of Fraenkel was employed to extract the dried gland, and the resultant waxy mass of extractives was washed several times with 85-90 per cent. alcohol. The alcoholic solution was precipitated with ether, the precipitate redissolved in hot alcohol, evaporated, and after it had crystallized, dissolved in distilled water and administered through the stomach tube.

The widening of the palpebral fissure can easily be made out in the photograph. (See text, p. 211).

Plate XV.

Photo-micrographs of small aortic accessory thyroid bodies.

Fig. 1. A longitudinal section of an accessory aortic thyroid body (×24 diams.) from a normal dog.

In the more perfectly developed portions the small vesicles contain colloid.

Fig. 2. A more highly magnified view (×300 diams.) of a part of a section of the preceding accessory body; the structure of a portion containing no colloid is shown. The appearance is similar to that of the parathyroid body.

Plate XVI.

Fig. 3. Cross-section of an aortic body embedded in a mass of fat, from a thyroidectomized dog that had a moderate dyspnœic attack on the third day after the operation. The dog was then injected with concentrated thyroid bouillon for several days and the injections then stopped, but no return of the symptoms occurred; the dog was killed by an over-dose of ether at the end of eight days.

In the cross-section a rather unusual amount of fibrous tissue is seen between the vesicles containing colloid. Hardening in alcohol, stained with hæmatox. and eosin, and magnified 24 diams.

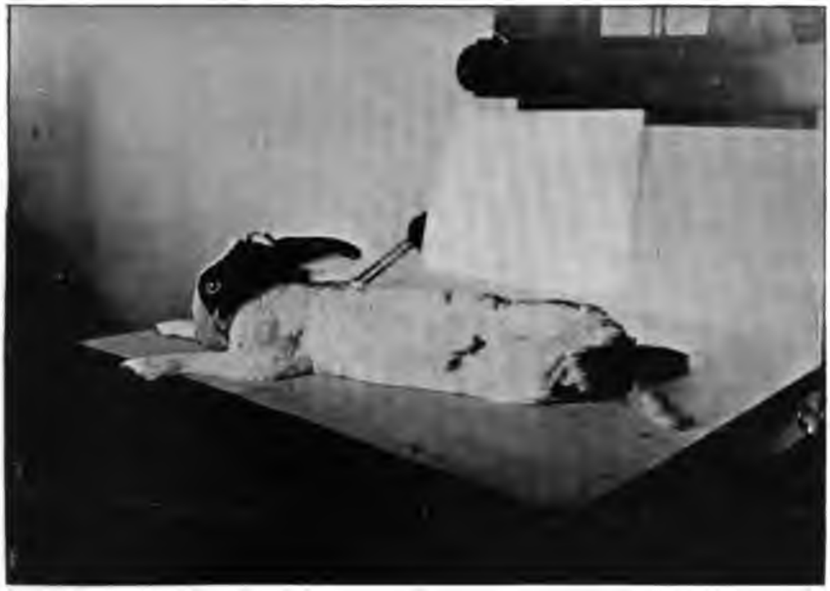
Fig. 4. Aortic body from a dog in which operative myxœdema had been produced by ligating all the thyroid vessels. As the myxœdematous swelling was beginning to subside an over-dose of ether was given and this with two other small aortic bodies was removed.

The structure of this body does not differ from that of the main thyroid lobes. (×24 diams.).

Plate XVII.

These tracings show graphically the cardiac and blood-pressure effects that follow the intravenous injection of thyroid and other extractives; in each of the tracings the top line is that of the blood pressure, the middle line is the base line, the bottom line is the time. At — the injection was made.

Fig. 1. Showing in an etherized dog the fall of blood pressure from an injection into the ext. jugular vein of 0.5 ccm. of a *neutral* 20 per cent. aqueous solution of the extractives from fresh raw thyroid. The brownish wax yielded by the acetate of lead method of Fraenkel was dissolved in 85 per cent. alcohol; the alcoholic solution precipitated by ether; the precipitate redissolved several times in boiling 90 per cent. alcohol, and several times reprecipitated by ether. Finally this precipitate was dissolved in a small quantity of distilled water; the solution evaporated on a water bath at 100° C., and then set aside to crystallize over chloride of calcium. This substance, which should be the same as Fraenkel's thyreo-antitoxin, was dissolved in normal saline and injected, producing the result seen in the figure.



24[?]

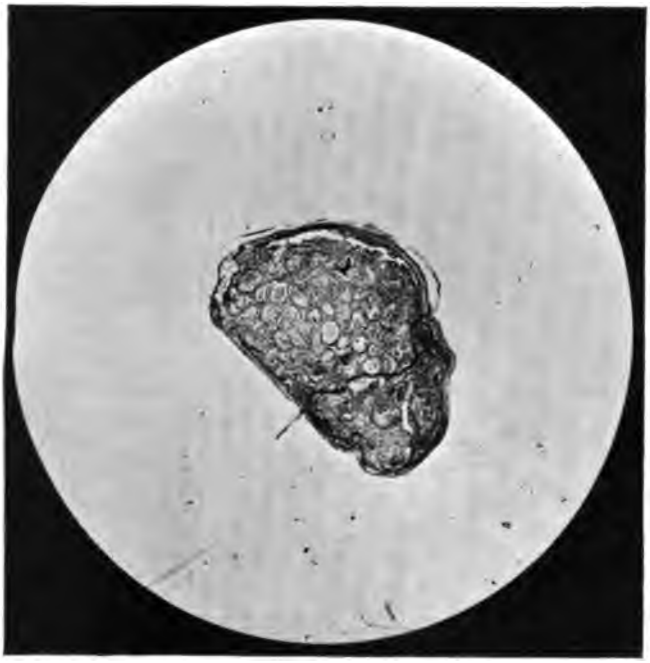


FIG. 1.

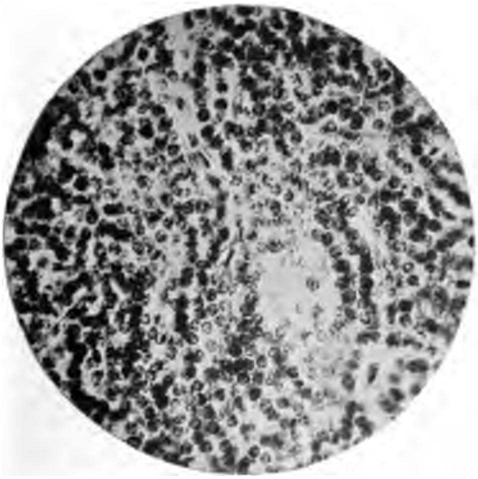


FIG. 2.

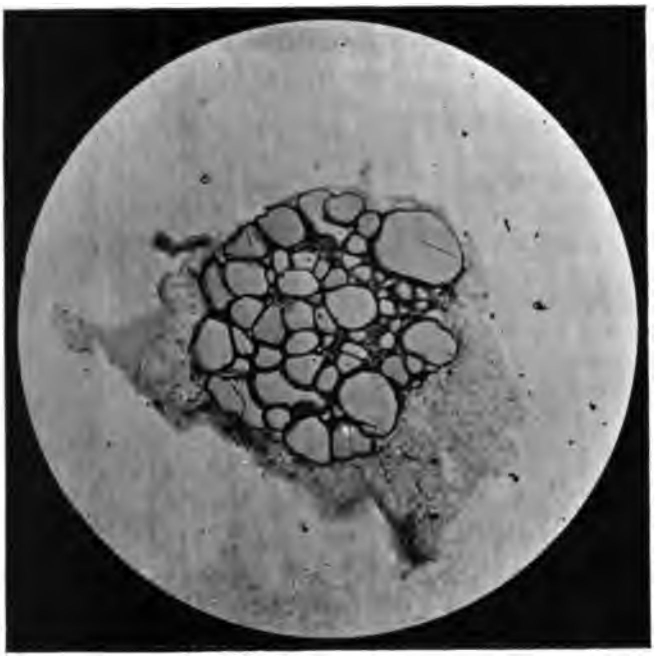


FIG. 3.

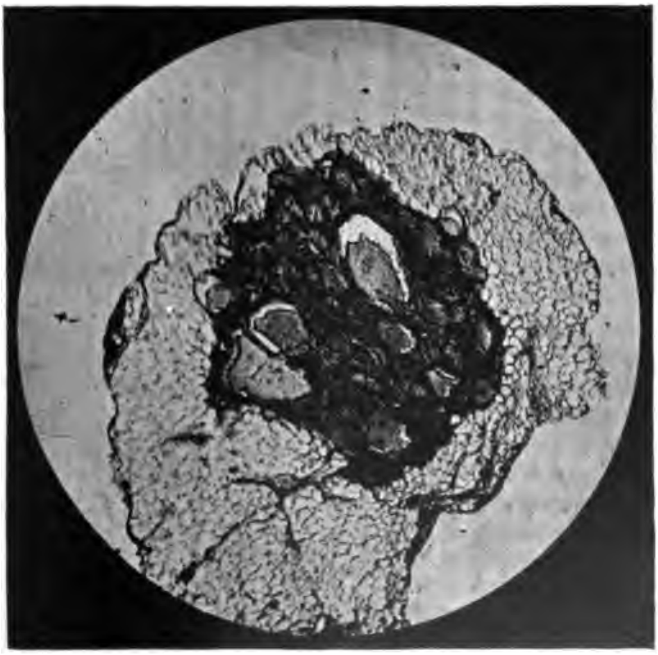


FIG. 4.

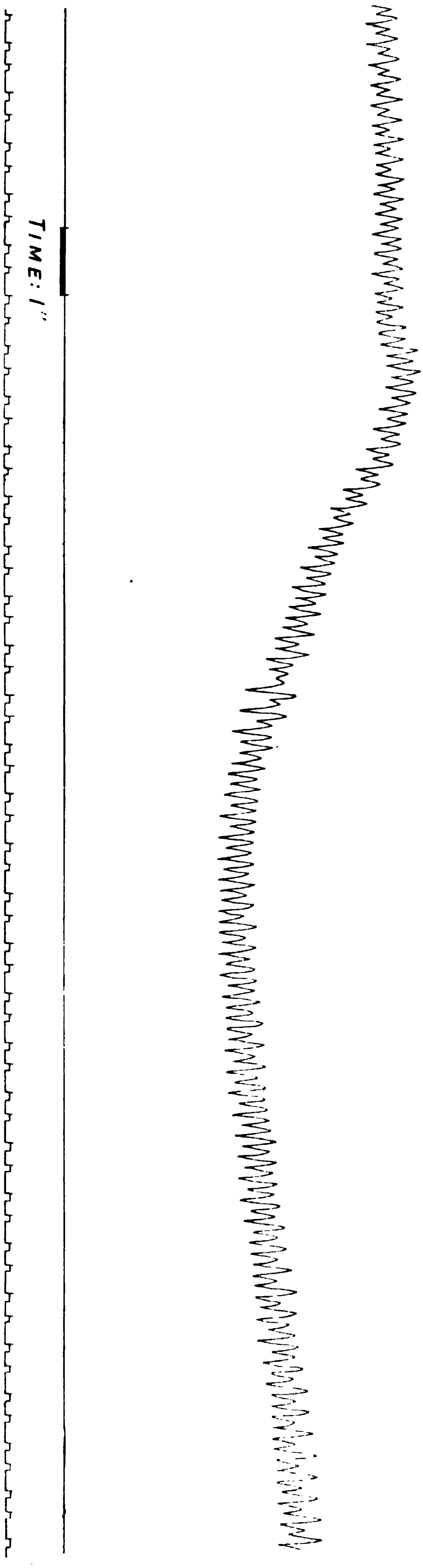


FIG. 1

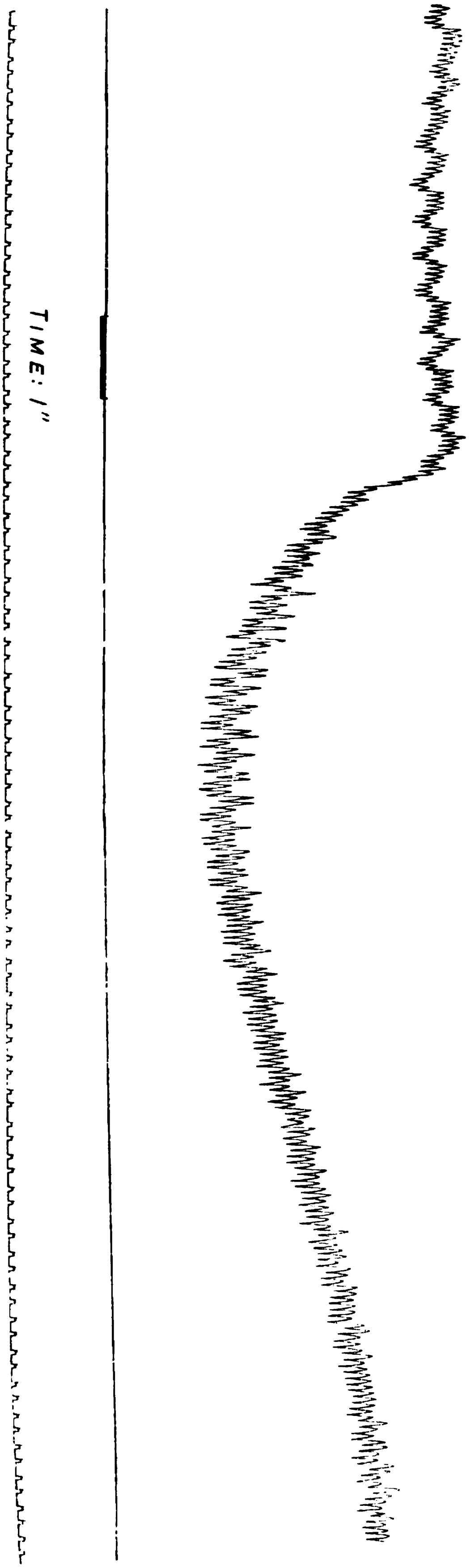


FIG. 2

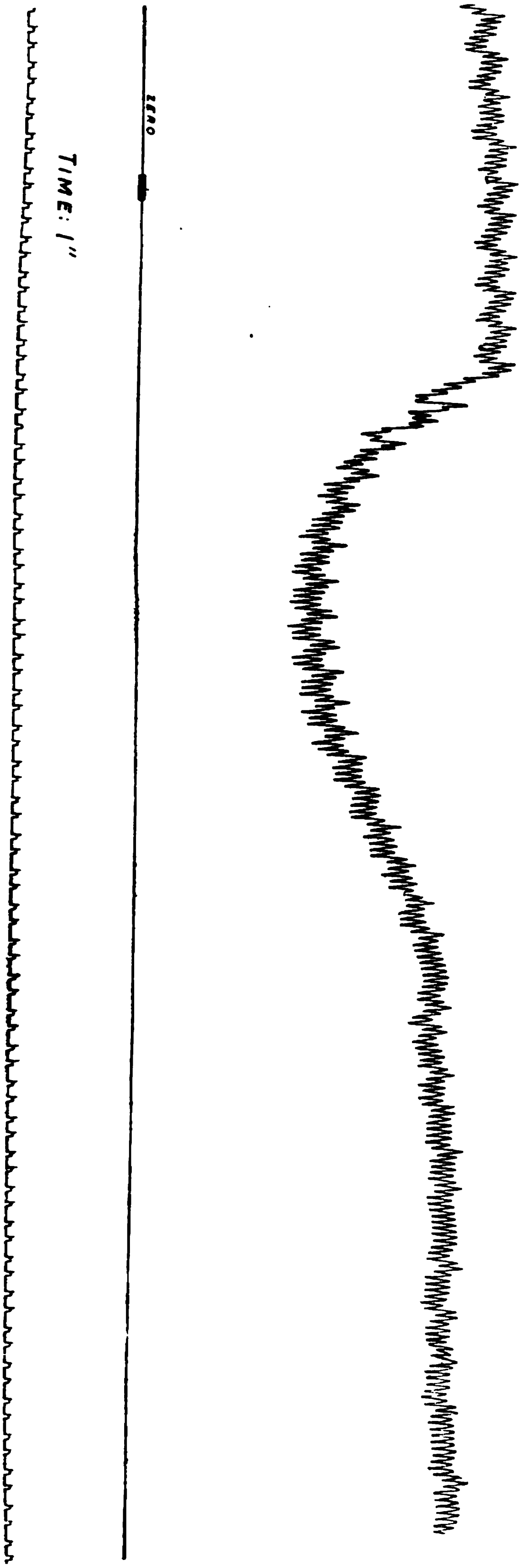


FIG. 4

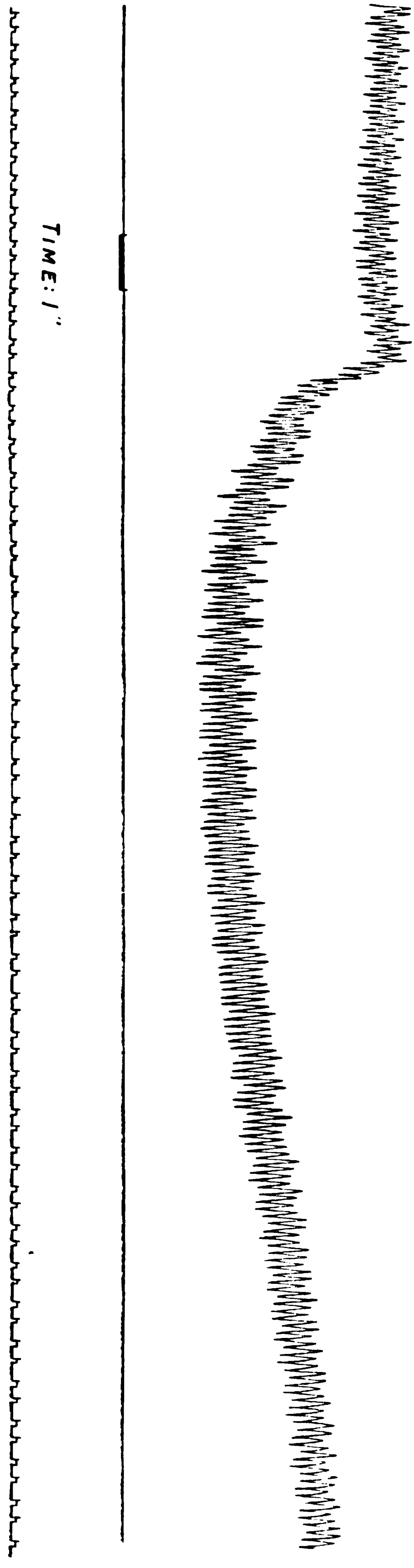


FIG. 3

Fig. 2. Showing after division of both vagi the effects in an etherized dog of 2 ccm. of a neutral 10 per cent. solution of a mixture of the two partially crystalline products obtained by treating the above waxy mass of fresh thyroid extractives with phospho-tungstic acid, etc., according to the method that Drechsel recommends for the fresh raw thyroid gland. Both the crystalline products had been repeatedly cleaned by the alcohol-ether method.

Plate XVIII.

Fig. 3. Showing the effects of injecting 2.5 ccm. of a 10 per cent. solution of a partially crystallized *thymus* product. The fresh thymus glands were extracted by the acetate of lead method and the resultant waxy mass precipitated by phospho-tungstic acid. After boiling this precipitate for a number of hours, decomposing the decoction with baryta, etc., and finally cleaning with alcohol and ether, the partially crystallized product used in this instance was yielded. Morphine was given to this etherized dog.

Fig. 4. The fall of blood pressure produced by 1 ccm. of the neutralized mother-liquor from which the creatin and creatinine had been removed. Liebig's extract of meat was the source from which this mixture was obtained, and the acetate of lead method was employed to extract the creatin.

THE PHYSIOLOGICAL EFFECTS OF EXTRACTS OF THE HYPOPHYSIS CEREBRI AND INFUNDIBULAR BODY.

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(From the Physiological Laboratory of the Johns Hopkins University.)

Injections of extracts of the hypophysis cerebri have been tested by Szymonowicz and by Oliver and Schäfer during the course of their well-known experiments upon the effects of extracts of the adrenal glands. Szymonowicz * states that in two experiments of this kind, made upon dogs, he obtained a slight fall of blood pressure and a quickening of the heart beat, just the reverse of the cardiac and vascular phenomena observed after injection of adrenal extracts. He concluded, therefore, that the physiological effects of extracts of the hypophysis cerebri are different from those caused by extracts of the adrenal glands, and, so far as can be gathered from his brief description, are much less marked. Oliver and Schäfer † obtained different results. In their hands extracts of the hypophysis caused usually a marked rise of blood pressure together with an augmentation of the force of the heart beat, results resembling those obtained from extracts of the adrenal body. There was, however, this difference, that the pituitary extracts, unlike the adrenal, caused no slowing of the heart beat. As these results are quite unlike those obtained from extracts of the thyroid body, the authors use them somewhat hastily, as appears from my experiments, to combat the prevalent view of a physiological relationship between the thyroid and the hypophysis cerebri. The contradictory results obtained by the investigators quoted suggested to me the desirability of making further experiments of the same nature upon this organ. The results of these experiments have been very uniform so far as the general effects of the extracts are concerned, and in some respects are quite different from those referred to briefly above.

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* Szymonowicz, Pflüger's *Archiv*, lxiv (1896).

† Oliver and Schäfer, *Journal of Physiology*, xviii (1895).

The hypophysis cerebri is usually described as consisting of two lobes. One, the large anterior lobe, is distinctly a glandular structure, as shown by its histological characteristics. Embryologically it is described as arising in part or entirely from the epithelium of the mouth cavity. According to Haller * it possesses an incomplete system of ducts that open between the meningeal membranes. Any secretion that it may form passes therefore, in part at least, into the cerebrospinal liquid contained between these membranes. Properly speaking the term hypophysis cerebri should be restricted to this lobe, and this significance is now given to it by morphological writers, although in human anatomy it is still commonly employed to include the so-called posterior lobe as well. The posterior lobe of human anatomy lies upon and is partially enclosed by the anterior lobe or hypophysis proper. It is connected by a stalk with the tip of the infundibulum, and embryologically it is to be regarded as an outgrowth from this part of the brain. It would seem more desirable therefore to speak of this body not as the posterior lobe of the hypophysis cerebri, but as the infundibular body, and this designation I shall use in this paper. The infundibular body is quite small as compared with the hypophysis. Its histological structure in the adult mammal has evidently not been studied very exhaustively. According to the older accounts it consists largely of a network of neuroglia, and it has been commonly regarded therefore as a rudimentary organ without distinct physiological value. Berkley † has studied its histology by means of the Golgi method and reports that its structure is complicated and somewhat curious. He finds in it numerous nerve cells belonging to several types, a cortical layer of ependymal cells, a network of neuroglia tissue, some peculiar structures resembling nerve end-organs, and lastly a quantity of glandular-like epithelial cells arranged in part to form tubes or closed vesicles, some of which contain a colloidal material. This rather complex structure indicates that the infundibular

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* Haller, *Morphologisches Jahrbuch*, xxv (1896).

† Berkley, *The Johns Hopkins Hospital Reports*, iv (1895).

lobe is not merely a rudimentary organ, and the glandular tissue that Berkley describes suggests a secretory activity of some kind. This suggestion I may say at this point is corroborated by the physiological experiments described in this paper.

In my experiments extracts were made of both the hypophysis cerebri, or anterior lobe, and the infundibular body, and the effects of each were tested separately by injection into the circulation of anæsthetized dogs. The extracts in some cases were made from the dried tissue prepared according to the method described by Oliver and Schäfer. That is, the glands were pressed out as thin as possible and dried rapidly in a sulphuric acid chamber. The dry material when used was powdered and extracted with a few cubic centimetres of normal saline solution. In most cases, however, glycerine extracts were made from the fresh tissues. In making these extracts the two lobes were first separated, each was then rubbed to a creamy pulp in a mortar with a small quantity of glycerine, and the mixture was allowed to stand for several hours. Before using this extract it was diluted with a few cubic centimetres of normal saline solution and was filtered either at once or after standing an hour or two.

A few experiments were made with the hypophysis of the dog, but in most cases the gland from the sheep was used, since it is much larger and can be obtained easily from animals killed at the slaughter-houses. In the sheep the hypophysis and the infundibular lobe together make a structure of considerable size, lying in the sella turcica and nearly covered by a bony and membranous operculum. This position makes its removal a simple matter, since the brain may be removed rapidly in mass from the skull without disturbing the hypophysis, the only precaution to observe being to first cut the stalk connecting the infundibular lobe to the infundibulum. The size of the entire organ in the sheep is variable, being proportional apparently to the size of the animal; the weight is usually from 300 to 500 mgrms. The hypophysis proper is much the larger of the two lobes. It forms a firm reddish mass incompletely separated on the upper side into two lobes by a shallow longitudinal furrow. The infundibular body is a small whitish mass weighing about $\frac{1}{10}$ as much as the hypophysis. It lies upon the upper surface of the hypophysis at its posterior angle, and the long stalk connecting it with the infundibulum runs in the shallow furrow that separates the two halves of the hypophysis. If this stalk is seized with a pair of forceps and dissected carefully backwards, it and the adherent infundibular body may be separated easily from their loose attachment to the hypophysis.

The extracts made from these two lobes were injected directly into the jugular or femoral vein of an anæsthetized dog by means of a fine-pointed hypodermic syringe, and as a rule 2 cc. of the extract were used for each injection. The effects obtained were as follows:

The extracts of the hypophysis alone caused little or no perceptible change in blood pressure or heart rate. In some cases the effect was entirely negative, in others a slight but inconstant effect upon blood pressure was noticed. Upon the whole it would seem that extracts of this body have no characteristic effect upon the circulatory or respiratory organs.

With the extracts of the infundibular body, however, the results were very marked and in their main features quite constant, although somewhat variable in detail and apparently slightly different according to the anæsthetic employed. The main effect upon an animal with its vagi intact was a pronounced slowing of the heart beat together with an increase in blood pressure; while upon an animal with its vagi cut or under the influence of atropin the effect consisted chiefly in a marked prolonged rise of blood pressure together with a slower and stronger heart beat. These two effects are illustrated in the accompanying illustrations (Figs. 1 and 2). The details of the curves obtained varied somewhat for each animal. In general the course of the curve when the vagi were intact was as follows: Within a few seconds (5-10) after the beginning of the injection the blood pressure rose to a variable extent; this was followed quickly by a temporary fall, which was also quite variable in amount. During this period the heart beat was somewhat slower and apparently more feeble, except in the case of peptone anæsthesia, in which on the contrary the heart rate was accelerated temporarily. The duration of the fall in pressure was also variable; in some cases it lasted for a few seconds

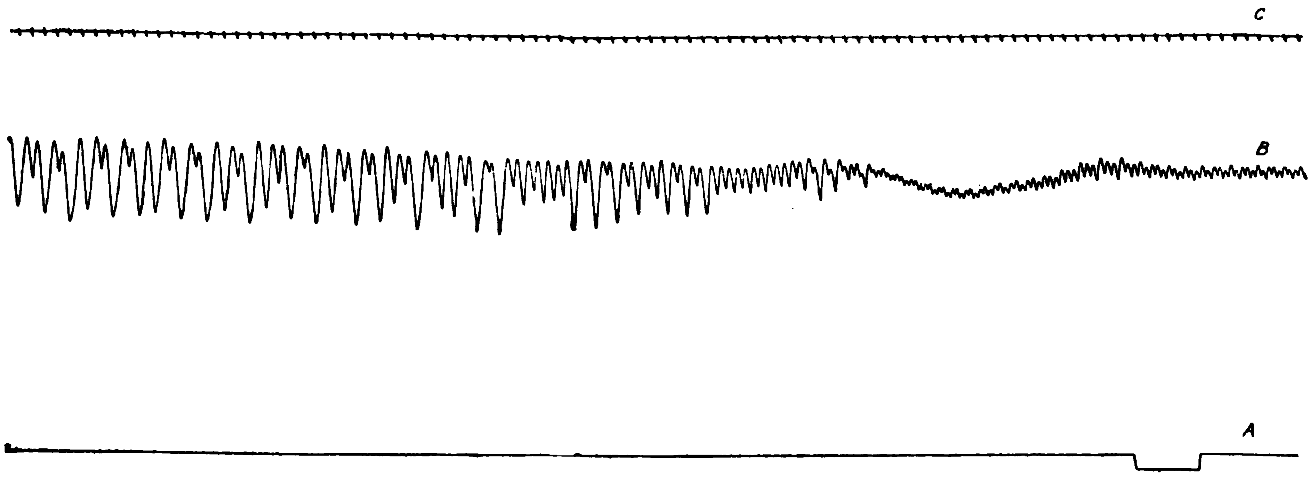

FIG. 1.

Tracing from a dog under peptone, showing the effect of extracts of the infundibular body upon the heart beat and blood pressure when the vagi are intact. The tracing reads from right to left and is reduced ½ in size. *A* is the base line. The depression in this line indicates the time during which the injection was made into the jugular. *B* is the blood pressure record; *C*, the time record in seconds. Before injection the blood pressure = 150 mms., the pulse rate = 144. During the first small fall in pressure the pulse rate = 165. At the end of the tracing the pulse rate = 55.5, and the maximal blood pressure = 163 mms.

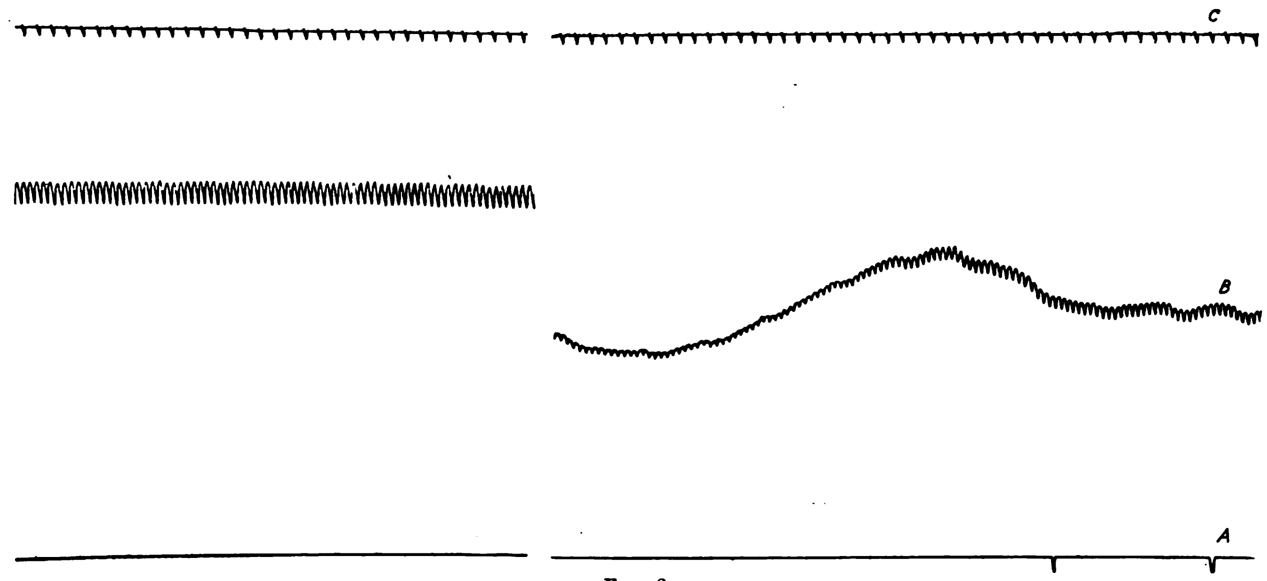

FIG. 2.

Tracing from a dog under morphia (and ether), showing the effect of extracts of the infundibular body when the vagi are cut. This tracing reads from right to left, and is reduced ½ in size. *A* is the base line; the injection of extract was made in the interval between the two marks on this line. *B* is the blood pressure record; *C*, the time record in seconds. Before injection the arterial pressure = 184 mms., the pulse rate = 151. The first rise of blood pressure reached to 162 mms., and in the succeeding fall the pressure sank to 108 mms., with a pulse rate of 147. At the end of the tracing the characteristic and constant effect of the extract is shown. The blood pressure here = 192 mms., and the pulse rate = 180.

only, while in one instance it continued for nearly a minute. This phase was then succeeded by the main effect, namely, a very marked slowing of the pulse rate that lasted for a long time, in some cases over half an hour. The maximum slowing was attained gradually, and the return to the normal rate was made still more slowly. During this period the maximum blood pressure first increased slowly, rising usually to a level above that prevailing at the time of the injection, and then slowly dropped back to normal. Roughly speaking the maximal blood pressure was reached at the time that the pulse rate was the slowest.

Some idea of the extent of these changes in pressure and pulse rate when the vagi are intact may be obtained from the following tabular statement of the results of some of the experiments:

Exp. 1. Dog under morphia (0.05 grm.) with a little ether.

Pressure before injection of extract of the infundibular body = 109 mms. Hg.
Greatest pressure after injection of extract of the infundibular body = 156 " "
Pulse rate per minute before the injection = 134 beats.
Slowest pulse rate per minute after the injection = 57 "

Exp. 2. Dog under morphia (0.05 grm.) with a little ether.

Pressure before injection of extract of the infundibular body = 120 mms. Hg.
Greatest pressure after injection of extract of the infundibular body = 160 " "
Pulse rate per minute before the injection = 68 beats.
Slowest pulse rate per minute after the injection = 33 "

Exp. 3. Dog under morphia (0.05 grm.) with a little ether.

Pressure before injection of extract of the infundibular body = 124 mms. Hg.
Greatest pressure after injection of extract of the infundibular body = 147 " "
Pulse rate per minute before the injection = 122 beats.
Slowest pulse rate per minute after the injection = 74 "

Exp. 4. Dog under morphia (0.05 grm.) with a little ether.

Pressure before injection of extract of the infundibular body = 113 mms. Hg.
Greatest pressure after injection of extract of the infundibular body = 148 " "
Pulse rate per minute before the injection = 93 beats.
Slowest pulse rate per minute after the injection = 40 "

Exp. 5. Dog under morphia (0.05 grm.) with a little ether.

Pressure before injection of extract of the infundibular body = 120 mms. Hg.
Greatest pressure after injection of extract of the infundibular body = 140 " "
Pulse rate per minute before the injection = 100.5 beats.
Slowest pulse rate per minute after the injection = 49.5 "

Exp. 6. Dog under peptone injected into the femoral artery, 1 dgrm. to 1 kilo.

Pressure before injection of extract of the infundibular body = 87 mms. Hg.
Greatest pressure after injection of extract of the infundibular body = 148 " "
Pulse rate per minute before the injection = 144 beats.
Slowest pulse rate per minute after the injection = 78 "

Second experiment upon the same animal:

Pressure before injection of the extract = 150 mms. Hg.
Greatest pressure after injection of the extract = 163 " "
Pulse rate per minute before the injection = 144 beats.
Slowest pulse rate per minute after the injection = 56 "

Exp. 7. Dog under ether alone.

Pressure before injection of extract of the infundibular body = 144 mms. Hg.
Greatest pressure after injection of extract of the infundibular body = 172 " "
Pulse rate per minute before the injection = 142 beats.
Slowest pulse rate per minute after the injection = 108 "

It will be seen from these figures that the injections increased the blood pressure from 9 to 42 per cent., while the pulse rate, omitting experiment 7, suffered a reduction of from 40 to 60 per cent. In experiment 7, in which the animal was deeply under ether, the reduction in pulse rate was only 24 per cent., a result approximating, as we shall see, that obtained from animals with the vagi cut. Deep anæsthesia with ether apparently suspended in part or entirely the normal irritability of the inhibitory centre. As compared with adrenal extracts the effect of extracts of the infundibular body is characterized by its long duration. The blood pressure rises more slowly to a maximum, remains above the normal level for a longer time and sinks quite gradually. The long continuance of the heart effect is even more marked, and it is curious that this effect should have been overlooked by previous observers. It is possible of course that in the experiments of Oliver and Schäfer and of Szymonowicz upon the pituitary gland, the extracts contained little or no material from the infundibular body, since no attempt was made in their experiments to distinguish between the effects of the two lobes. The slowing of the heart comes on somewhat later than the effect upon blood pressure; it increases to a maximum within one or two minutes after the injection, and then decreases very gradually, the pulse rate returning to the normal at the end with some suddenness. The entire effect may last for twenty or thirty minutes or longer when the dose is a maximal one. If, however, repeated injections are given the effects caused by the successive injections may be much less marked or may fail altogether, the result depending upon the interval of time between the injections and also upon the strength of the dose used. The loss of reaction following upon repeated injections seems to be much more marked than in the case of adrenal extracts.

A typical example of the effect of repeated injections may be given in detail to illustrate the disappearance of the reaction even with maximal doses and with intervals of time sufficient or nearly sufficient for the effects of preceding injections to pass off. In Experiment 6 of the above summary the following results were obtained. Previous to the injection of peptone the blood pressure had been 144 mms., and the pulse rate 165.5 per minute. After injecting peptone rapidly into the femoral artery, one decigramme to a kilo of animal, the pressure sank rapidly to 28 mms., while the pulse rate rose to 216. An interval of half an hour was allowed, and at the end of this time the pressure was 87 mms., while the pulse rate was 144. Several successive injections of extracts of the infundibular body were then made.

The effect of the first injection:

The pressure was raised to 148 mms.
The pulse rate was slowed to 78 beats.

Twenty minutes after this injection the pressure was still at 150 mms., while the pulse rate had returned to 144.

Effect of the second injection twenty minutes after the first:

The pressure was raised to 163 mms.
The pulse rate was slowed to 55.5.

At the end of seven minutes the pressure had returned to 152.5 mms., and the pulse rate to 115.5.

Effect of the third injection seven minutes after the second:

The pressure was raised to 161 mms.
The pulse rate was slowed to 69.

At the end of four minutes the pressure had returned to 144 mms., and the pulse rate to 139.5.

Effect of the fourth injection four minutes after the third:

The pressure was raised to 148 mms.
The pulse rate was slowed to 102.

The effect in this case was quite transient, the heart rate and blood pressure returning to normal within one minute.

It would seem from this and similar experiments that the active substance of these extracts, as in the case of the adrenal extracts, is destroyed or neutralized in some way in the body so that its action is comparatively temporary. For this reason also injection of relatively large quantities of the extract when made subcutaneously fails to give any reaction upon the heart or blood-vessels, none, at least, sufficiently distinct to be detected with certainty upon the kymographic records. It would seem that an injection of this extract made directly into the circulation leaves the heart and blood-vessels in a condition of diminished irritability toward a new injection. This loss of reaction is certainly not due to a paralysis of the muscles of the circulatory organs or the nerves supplying them, since repeated injections of maximal doses cause no depression of vascular tone, nor removal of the normal tonic inhibition of the heart. The blood pressure and pulse rate in such cases simply return more or less completely to the normal, and new injections have either a negative result entirely or an effect more temporary than that of the preceding injections. It has seemed in some experiments as though repeated injections resulted in a permanent improvement of vascular tone and a slower and stronger heart beat, but as this effect, when observed, came on quite slowly, it was not possible to determine satisfactorily whether it was due to the injections. All that can be said positively is that the sudden reaction upon the pulse rate and blood pressure ceases after renewed injections, occurring at short intervals, without any appearance of fatigue or paralysis in the heart or blood-vessels. These organs, on the contrary, seem to acquire an immunity from the active substance of the extract. How long this immunity may last was not determined.

The effects of the injections when made upon animals with the vagi cut or under the influence of atropin differed from those described above in that the blood pressure was increased to a greater extent, while the slowing of the heart beat was much less marked. The fact that the heart rate was slowed at all under these conditions is very interesting and constitutes a marked difference between the effects of extracts of the infundibular body and the adrenal gland. This fact indicates moreover that extracts of the infundibular body affect the heart in part by acting on the cardio-inhibitory centre, and in part by a direct peripheral action exerted either upon the heart musculature directly or upon its intrinsic nerves. The general character of the results obtained when the connections of the heart with the inhibitory centre are removed may be illustrated by the following summary of experiments:

Exp. 1. Dog under morphia (0.05 grm.) with a little ether. Vagi cut.

Pressure before injection of extract of the infundibular body = 92 mms.
Greatest pressure after injection of extract of the infundibular body = 180 "
Pulse rate per minute before injection = 148 beats.
Slowest pulse rate per minute after injection = 93 "

Exp. 2. Dog under morphia (0.05 grm.) with a little ether. Vagi cut. Two previous injections with the vagi intact.

Pressure before the injection of extract of infundibular body = 128 mms.
Greatest pressure after the injection of extract of infundibular body = 145 "
Pulse rate per minute before injection = 144 beats.
Slowest pulse rate per minute after injection = 120 "

Exp. 3. Dog under morphia (0.05 grm.) with a little ether. Atropin injected, vagi cut.

Pressure before injection of extract of infundibular body = 134 mms.
Greatest pressure after injection of extract of infundibular body = 192 "
Pulse rate per minute before injection = 151 beats
Slowest pulse rate per minute after injection = 124.5 "

In these experiments the increase in blood pressure varied from 13 to 95 per cent. of the original pressure, while the reduction in pulse rate varied from 17 to 35 per cent. The comparatively small effect upon blood pressure in experiment 2 is probably due to the fact that two injections of extract had been made before the section of the vagi, and, as was stated above, repeated injections are followed by a smaller reaction even when a considerable interval of time is allowed to intervene.

I have not in these experiments made any very thorough attempt to determine whether the extracts of the infundibular body affect the musculature of the heart and blood-vessels directly or act mainly upon the central nervous system. Two experiments, however, in this direction have yielded results that indicate that the active substances in these extracts, as in those of the adrenal glands, cause a rise of blood pressure by peripheral action upon the blood-vessels. In one experiment the spinal cord was cut below the medulla, and in addition most of the thoracic and lumbar cord was extirpated. After this operation the blood pressure had fallen to 23 mms. Hg, and the pulse rate per minute was 146. Injection of 2 cc. of an extract of the infundibular body caused the pressure to rise to 72 mms., an increase of 213 per cent., while the pulse rate fell to 91 beats, a reduction of 37 per cent. This experiment indicates that if the rise of blood pressure was caused by a peripheral constriction of the arteries, this constriction was not due to an effect upon the spinal centres, as claimed by Szymonowicz in the case of adrenal extracts, but to an action upon the arteries themselves. In a second experiment the kidney was placed in an oncometer and its changes in volume were recorded together with the kymographic tracing of the pulse and blood pressure. It was found that the volume of the kidney decreased as the blood pressure rose and vice versa; thus indicating that the increase in blood pressure is accompanied by and in part most probably caused by a peripheral constriction of the arteries.

At various times during these experiments I have tested the effect of glycerine extracts of different parts of the central nervous system, especially of the cerebral cortex, the medulla oblongata and the pineal gland. The extracts of the three last-named bodies have given inconstant results, which may be accounted for partly perhaps by the fact that the injections were made usually in the course of experiments upon the extracts of the infundibular body. In some cases the results of these injections were negative or very slight, but at other times they caused a marked fall in blood pressure. Their effect certainly differed very greatly from the constant and characteristic reaction caused by the extracts of the infundibular body, and it seems quite evident that this structure contains a material distinct from anything occurring in other parts of the brain.

What this substance is I have not as yet attempted to determine. Its physiological effects resemble closely those caused by the active substance in adrenal extracts, but, as has been stated above, it differs from the adrenal extracts in the marked slowing of the heart beat that it causes after the two vagi have been cut, as well as in the longer duration of its action both upon the heart beat and the blood pressure. Moreover, the physiological reaction of the extracts of the infundibular body differs very distinctly from that caused by extracts of the hypophysis cerebi. This fact, taken in connection with the difference in structure of the two bodies and their difference in embryological origin, seems to indicate that physiologically as well as anatomically they are independent structures. The method of injecting extracts appears to teach us nothing with regard to the physiological activity of the hypophysis, it neither confirms nor disproves its supposed relationship to the thyroid body. On the contrary the marked influence of extracts of the infundibular body suggests that this organ may form a secretion of great importance to the functional activity of the circulatory organs. Certainly the effects caused by its extracts tend to disprove the prevalent view that this body is merely a functionless rudiment of an organ of primitive importance.

Haller,* in his interesting paper upon the development of the hypophysis, points out that in some of the lower vertebrates, the teleosts for example, a true glandular organ, the infundibular gland or saccus vasculosus, develops as an outgrowth from the infundibulum and maintains an open connection with the infundibular cavity. He concludes therefore that in these forms the gland opens directly into the cerebral ventricles. In the higher vertebrates, the mammals for instance, he finds no trace of this gland, but instead a solid outgrowth, which he calls the processus infundibuli, attached to the tip of the infundibulum and apparently rudimentary in structure. This processus infundibuli corresponds apparently to the infundibular lobe, the effect of whose extracts has been described in this paper, and it seems possible that the solid process still retains the glandular structure of the primitive infundibular gland.

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* Haller, loc. cit.

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A TERATOMA OF THE ABDOMINAL CAVITY.

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PLATES XIX-XXII.

Teratomata have always attracted attention by their strange appearance, but of late years they have given rise to much investigation on account of the promise they hold out of elucidating the etiology of the true tumors.

The tumor to be here described was a solid teratoma of the abdominal cavity, in itself a sufficiently rare occurrence, and, besides, was remarkable on account of the great variety of tissues which it contained. Clinically also it took an unusual course, for, after being operated on and apparently entirely extirpated, it rapidly recurred.

In May, 1893, Dr. W. F. McNutt was called by Dr. J. S. Stone to see a patient, a girl, 12 years of age, who was suffering from an abdominal tumor. The patient herself had noticed ten months previously that she was growing stouter and that her clothes no longer fitted her. The rate of growth had at first evidently been slow, but latterly very rapid, as Dr. Stone for a few days previous to the consultation had been able to make out an increase in girth of one and one-half inches a day. Both previous to and after the enlargement of the abdomen the patient had always enjoyed good health. There was no loss of flesh and she had never suffered any pain. There was nothing in the family history to indicate any hereditary inclination to the formation of neoplasms. One grandfather had died of tuberculosis. There was no history of multiple pregnancies on either side of the house.

On physical examination the abdominal veins were found dilated and the abdomen was considerably enlarged. The swelling was general, and much of it was evidently due to ascites. By palpation and percussion a solid mass could be made out in the right side of the abdomen.

On May 27, 1893, the abdomen was opened, and the tumor, together with about two gallons of slightly bloody ascitic fluid, was removed.

When Dr. McNutt brought me the specimen he said that the tumor was found on the right side of the abdomen and in the peritoneal cavity; that its general shape, as it lay in the abdomen, was long, narrow and curved, and that the shorter arm of the curve lapped around the caput coli, while the longer arm extended up along, and was attached to, the outer side of the ascending colon throughout nearly its whole length to a point nearly as high as the liver; that it shelled out easily, and when it was taken away the operation seemed to be complete, as there was no appearance of any other diseased tissue in the abdominal cavity. Dr. McNutt also told me that in removing the tumor no large blood-vessels were encountered, the vessels being only such as run in brittle adhesions; and while that part of the peritoneum from which the growth was detached was of course raw and bleeding, the rest of the peritoneal surface, as far as could be seen, was perfectly smooth, glistening, and normal. After the operation the abdominal cavity was closed and the patient made a speedy and uneventful recovery for the time being.

A photograph of the tumor is shown in Plate XIX, Fig. 1. When I first saw the original tumor, a few hours after the operation, it lay in a basin, a formless, quivering, semi-fluid, jelly-like, shiny mass, with white, greasy patches on its surface, and small pieces of bone, the size of a silver half-dollar and less, scattered in its substance. After being in alcohol for some time the tumor became firmer and assumed more definite outlines. It weighed two pounds, and resembled very strikingly a cerebral hemisphere. Like this it was elongated and lobulated, and all the lobules were smooth and grew out in one direction as if they sprang from a common elongated base. Two of the fissures cut into the tumor so deeply that they divided it into three parts, a large central portion with a smaller portion at each end. These three lobes, besides being united at their bases, were connected together with strings of tissue thrown across the fissures. On cutting into the tumor it was soft, and in many places honeycombed with minute cysts, many of which were so large that they could be seen with the naked eye. The largest cyst was about the size of a cherry; then there were a number of pea-sized cysts, but by far the greatest number were very minute and could only be made out with a lens or a microscope. In spite of this cystic structure, however, the tumor, looked at as a mass, was a solid growth, a point of a good deal of importance considering the rarity of solid dermoids in the interior of the body.

The term solid teratoma or dermoid does not mean that the tumor does not contain cysts. In fact such teratomata contain large numbers of cysts, but these cysts do not interfere markedly with the general solid appearance of the neoplasm, while in the so-called cystic neoplasms of this class the hollow character of the tumor is the main feature.

Almost the whole of the outer surface of the tumor was covered with a distinct epithelial membrane. In many places this covering was clearly skin (Plate XIX, Fig. 2), being provided with fine downy hair and sebaceous glands. In other situations it was devoid of hair and looked more like the mucous membranes that are covered with flat, stratified epithelium. This epithelial covering, provided or unprovided with hair, followed the irregularities of the tumor down into the deepest fissures. On some parts of the free surface evidences of an epithelial covering were not distinct to the naked eye, but even in such places on microscopic examination there could be made out occasional indications of a papillary layer, or groups of cells indicating probably the remains of an epithelial covering.

In most dermoids the skin lines one or more of the cysts of which the tumor is composed; in this tumor, however, nearly all the cutaneous integument was found on its external surface, and only one small cyst was discovered lined by skin.

The extent of this cutaneous envelope, its position on the outside of the tumor, the ease and apparent completeness with which the tumor was shelled out of the peritoneal cavity, were noteworthy facts, making it seem probable that this teratoma was an independent individual enclosed, to at least a great extent, in its own skin.

Although a number of small pieces of bone were present in the tumor, none of them resembled at all clearly in shape any definite human bone. Nor did the islands of cartilage here and there met with (Plate XX, Fig. 4) show a form recognizable as that of any of the cartilages or bones of the body. There was nothing in the tumor therefore which could justly be called a skeleton, nor could it be made out, as is sometimes possible in teratomata, that the bones were arranged according to the conformation of the fœtal skull. But, as will be shown further on, one of the pieces of bone may have represented a fusion of some of the bones in the neighborhood of the ear. All the pieces of bone, as well as the islands of cartilage, were bound in the tumor by strong attachments, so that it was difficult to free them from the surrounding tissues. The largest piece of bone had a hollow in it enclosing an empty tube about half an inch in diameter. The total weight of the pieces of bone amounted to about one ounce. No teeth were found anywhere in the growth.

As has been said, in many places the tumor was honeycombed and spongy with minute cysts, but in other regions there were very few cysts, and in still other areas of considerable size there were none at all. Some of the cysts communicated with one another. The majority were filled with a mucoid substance, and none of them were filled with sebum. No polypoid or warty projections were found jutting into the cysts. Some of the cysts had a refractive yellowish lining as smooth as peritoneum, and one of them was found lined with skin.

The appearance of the tissue in among the cysts and between the bones and masses of cartilage varied considerably. In some places it was fairly dense and tough and apparently fibrous; in others, much softer and even jelly-like. In many parts of the stroma there were streaks of black or brownish-black pigment, and in one place a sheet of tissue, measuring 1 x 2 cm., covered on one side by a thin, continuous, even, black pigment layer, was found attached by one of its edges to a bone in the centre of a large lobe. The other relationships of this sheet of tissue could not be determined owing to the disturbance of the parts in getting out the bone. This sheet of tissue is interesting because, on microscopic examination, it was shown to be a part of an eye.

Pieces of the tumor, hardened in alcohol, were embedded in celloidin, sectioned and stained by various methods. A study of the finer structure of the growth revealed even more complicated relations than the gross appearances had indicated. The growth contained tissues and portions of organs corresponding in embryonic origin to all three of the germinal layers. Corresponding to the epiblast there were skin with cutaneous organs and appendages, central nervous system, peripheral nerves, and rudiments of eye structures. The hypoblast was represented by mucous glands, tubes and cysts with epithelial lining and surrounded by smooth muscle. The mesoblastic tissues consisted of bone, cartilage, white fibrous tissue, yellow elastic tissue, mucoid connective tissue, adipose tissue, smooth muscle fibre, and blood-vessels.

The best developed organ in the teratoma was the skin with its hairs and attendant sebaceous glands. This is a regular phenomenon in such tumors, and it is usually such a prominent feature as to give them the name dermoid. This predominant participation of the skin is supposed to be due to the fact that the ectoderm has the start of the other fœtal layers in development. The skin in the case under consideration was so far advanced that there was at any rate a commencement of defluvium of the hair (Plate XX, Fig. 6). The embryonic nervous system, the eye-structures, and structures belonging to the intestine were also remarkably developed. Microscopic examination of the skin of the surface of the tumor showed the presence of nearly all the constituents of normal skin—epidermis, corium, tela subcutanea, hairs with hair follicles, sebaceous glands, and blood-vessels. In one section of skin a definite bundle of mature medullated nerve fibres was found. As so frequently happens in dermoids, no sweat glands were discovered.

According to Wilms,* the presence of tissue of the central nervous system in dermoids seems to be a common occurrence, for of nineteen consecutive cases of dermoid cyst of the ovary examined by him he found such tissue in eighteen. Professor W. H. Welch first drew my attention to the presence of tissue of the central nervous system in this tumor, and its description will be left to Dr. Lewellys F. Barker (p. 284 et seq.).

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* Wilms, Ueber die Dermoidcysten und Teratome, mit besonderer Berücksichtigung der Dermoide der Ovarien, *Deutsch. Arch. f. klin. Medicin*, lv, 289.

It has been previously mentioned that a sheet of tissue was found covered by a thin, continuous, even, black layer, and attached by one of its edges to a bone in the centre of a large lobe. Transverse sections of this sheet of tissue showed the black layer to correspond to the pigment epithelium of the retina. There were flat polygonal cells filled, excepting the nucleus, with dark brown pigment, and these cells were so placed as to form a mosaic (Plate XXII, Fig. 11). This epithelium lay on a slightly pigmented connective tissue that was much firmer than the soft connective tissue usually encountered in this tumor. No definite tunica vasculosa (chorioidea) could be made out. The pigment epithelium could have been nothing but the lamina pigmentosa retinæ, as there is no other structure in the human body that resembles it; and the firm, slightly pigmented, in places distinctly laminated connective tissue on which it lay was probably the sclera.

Structures corresponding evidently to fœtal intestine were found. This could be seen as an oval tube cut transversely and lined with exquisite cuboidal epithelium. Numerous villus-like processes projected into the lumen. Beneath the epithelium there was a layer of cellular connective tissue, and the whole tube was surrounded by a definite tunica muscularis consisting of bundles of smooth muscle fibres, cut chiefly obliquely, but in part transversely (Plate XXI, Fig. 8).

Another tube lined with cylindrical epithelium lay close to a small mass of cartilage. This might have been embryonic trachea, though no smooth muscle could be made out in its wall, nor did the lining epithelium bear demonstrable cilia. Cysts with mucus in the lumen and lined by ciliated columnar epithelium with beautiful goblet cells were, however, also found, and doubtless corresponded to some part of the respiratory tract. Nothing recognizable as liver or pancreas was found.

The tube, which has already been mentioned as having been removed from a concavity in the largest piece of bone, and which was about 1 cm. long and 7.5 cm. in diameter, was examined microscopically. The wall of the tube contained a great deal of elastic tissue, the delicate fibrils of which were well brought out by the orcein stain. In some places the elastic tissue was arranged in the form of alveoli containing cartilage cells. There were also numerous large hairs in the wall of the tube. This tissue was therefore elastic cartilage, and as it was a tube situated in the hollow of a bone, and as there were also large hairs in the wall of the tube, this elastic cartilage may have been from the external ear. It is also of interest that this hollow bone with its tube containing elastic cartilage was situated near the sheet of tissue containing the flat pigmented epithelium of the eye already described. The bone was too large for the quadrate bone, but it may have represented a fusing together of several bones in the neighborhood.

The stroma of the tumor, made up of mesodermal structures, was not inferior to the epithelial elements in point of variety. There were little masses of hyaline cartilage showing concentric arrangement of cells toward the periphery, and each mass was surrounded by a well marked perichondrium; bone with bone corpuscles and canaliculi; mucoid connective tissue; white fibrous tissue; yellow elastic tissue; adipose tissue; embryonic connective tissue, resembling round-celled and spindle-celled sarcomatous tissue; and long bands of smooth muscle fibre. Then there was a great deal of intracellular and diffuse (intercellular) dark brown, granular pigment; and the tumor was well supplied with small blood-vessels, arteries, capillaries, and veins, having for the most part embryonic walls. No trace of a heart was found. The blood in the blood-vessels had all the characteristics of adult blood. No nucleated red blood corpuscles were seen. The white and red blood corpuscles appeared to be present in normal proportions.

No striated muscle fibre was found in the tumor. All the structures in the interior of the tumor were shuffled up together without any ascertainable law or order. There was an immense variety in the kind of tissue lining the cysts; some, as said, were clothed with flat, stratified epithelium; others with simple columnar epithelium; others with stratified columnar epithelium; others with ciliated columnar epithelium; and still others with goblet cells (Plate XX, Fig. 3). There were also many irregular cysts or alveoli containing stratified columnar epithelium forming wild-looking figures and broken festoons scattered about in a disorderly fashion in the alveoli, and without a basement membrane, as in adeno-carcinoma.

The cyst situated near the choroidal structure, before mentioned, was lined with skin provided with hair and sebaceous glands. This was the only unequivocal dermoid cyst within the tumor, as all the rest of the skin was on the outside of the tumor instead of lining a cyst. There were other cysts lined with flat stratified epithelium, but this, of course, might have been mucous membrane and not skin.

In many places there were masses and strings of round or polyhedral epithelial cells forcing their way between the fibres of the stroma of the tumor as in ordinary carcinoma (Plate XX, Fig. 7). There was also the formation of epithelial pearls and of onion-like bodies, which so frequently happens when flat epithelium grows in a confined space, as notably in flat-celled epithelioma.

Everything went well after the first operation for a few weeks, and the child appeared to enjoy her usual good health, but in July, a little over a month after the first operation, the abdomen was seen to be enlarging again.

A second operation was performed July 15, 1893, when a mass about as large as that removed at the first operation was taken away. At the first operation the tumor shelled out easily and the operation appeared to be complete. The second operation, however, Dr. McNutt told me, was manifestly incomplete. The friable, glairy, recurrent tumor, which was located mainly on the right side of the peritoneal cavity, had to be removed in pieces and could not be enucleated, and much of the diseased tissue could not be removed at all, as it had spread out laterally and involved a much larger surface of the peritoneum. At this operation a considerable quantity of bloody serum was found in the abdominal cavity, but not nearly so much as at the first operation. The abdomen was closed, and the patient died July 18, 1893, 51 days after the first operation.

After death Dr. McNutt made a simple incision into the abdomen and found that the diseased tissue had spread as a continuous layer of glairy, friable tissue over the visceral peritoneum of the lower part of the abdomen behind the bladder and nearly across to the left side of the abdominal cavity. The parietal peritoneum showed no evidence of disease. There did not seem to be any involvement of the intestinal wall excepting the growth of the neoplasm in the peritoneum covering the intestine. The abdomen only was opened and none of the viscera were cut into; the liver, the spleen, the kidneys, the uterus and ovaries were not incised, but they all appeared to be normal as far as could be judged from the external surface. The thoracic cavity was not examined. Very little fluid was found after death in the abdominal cavity.

The quick recurrence of the tumor *in loco*, its rapid growth, its spread by lateral extension, the appearance of the soft, glairy, brittle tissue removed at both operations, as well as the occurrence of bloody fluid in the peritoneal cavity in both instances, were enough to establish the identity of the recurrent growth and the original tumor, and the microscopical examination was confirmatory of this in every respect.

The general character of the recurrent growth, like the original, was that of a solid tumor richly studded with cysts, most of which were microscopic in size. A great many of the cysts were, however, as large as a pea, and some of them of the size of a walnut. As in the original tumor there was a great variety in the kind of tissue lining the cysts, and one cyst was sometimes clothed with many varieties of epithelium; for instance, columnar epithelium with nuclei far from the base of the cell was continuous with columnar epithelium with nuclei near the centre or near the base. This in turn was continuous with ciliated epithelium, which was finally continuous with round or polyhedral epithelial cells. As another example, an irregular cyst was lined on one side with stratified columnar epithelium, and the opposite side was covered with stratified flat epithelium, while a great number of flat epithelial cells were desquamated into this cyst cavity. In another cyst, stratified columnar epithelium tapered off into a single layer of cuboidal epithelium. There was a great number of well formed tubes lined entirely with stratified columnar epithelium. Many cysts were lined either partly or entirely with a single layer of beautiful columnar epithelium, each cell of which had a single nucleus placed far out at its distal extremity. Examples of this kind of epithelium were found both in the original and in the recurrent tumor, and were at first thought to represent the membrana adamantina of the fœtus, but a hair was discovered in the original tumor, in which the outermost cells of the external root sheath had exactly these characteristics; the cells formed very long columns, much longer than they usually do, and each cell was provided with a single nucleus that lay far out at its distal extremity (Plate XX, Fig. 5). Therefore, although the cells that looked like those of the membrana adamantina might really have been so, yet there was a possibility that they might have belonged to hair follicles where the development stopped with the formation of the external layer of the external root sheath. In further support of the pilary origin of these cells many follicles were detected having about the diameter of a hair follicle, and lined with a single layer of columnar cells, with their nuclei far out toward the free extremity of the cell.

The recurrent growth, like the original, contained tissues from all three of the germinal layers. Indeed nearly all the structures met with in the original tumor could be found in various parts of the tissue removed at the second operation. There were large masses of tissue of the central nervous system. The endodermal structures were scattered through the other tissues in considerable amounts, for instance, cysts lined with ciliated and columnar epithelium, and spaces lined with goblet cells.

The mesodermal stroma of the recurrent tumor was similar to that of the original, and consisted of little globes of hyaline cartilage surrounded by perichondrium, mucoid connective tissue, adipose tissue, well formed fibrous tissue, embryonic round-celled and spindle-celled connective tissue resembling sarcomatous tissue. One small flat piece of bone was removed with the tissue taken away at the second operation, but of this more hereafter.

In the immediate neighborhood of some of the tubes lined with lamellated epithelium, stratified columnar epithelium could be seen to form the wildest wreaths and broken festoons, scattered in irregular cavities much as in adeno-carcinoma. There were also places where masses of round epithelial cells could be seen invading the stroma of the tumor and pushing along between its fibres as in ordinary carcinoma. There was one particularly striking example of the metaplasia of columnar into flat epithelium (Plate XXI, Fig. 9). This was found where several follicles lined with simple columnar epithelium appeared to meet. The epithelial walls of two of these follicles were imperfect, and the epithelium at these imperfect points, as well as that lining the mouth of a third follicle, seemed clearly to have changed into flat epithelium with pearl formation. Of course there was a possibility that an invasion of flat epithelium might have taken place, but any one looking at the specimen would be surely drawn to the view that it was an instance of metaplasia of columnar to flat epithelium. Nests of flat epithelium containing pearls were found in the recurrent as well as in the original tumor (Plate XXI, Fig. 10).

That this tumor was a teratoma there can be no doubt, as is shown by the variety of tissues entering into its structure. It being settled that the tumor was a teratoma, the determination of the exact locality in which it grew is the next point to be considered.

As mentioned by Ernst Schreiber,* teratomata are oftenest found in the region of the head or sacrum. The next most frequent locality for their growth is the genital glands, and therefore the possibility of the tumor under discussion having originated in the ovaries must be carefully considered. But decidedly against this view is the fact that the growth lay high up in the abdominal cavity, and well away from the ovaries, which were found perfectly healthy and in their usual situation. Furthermore, no strands of tissue binding the tumor to the ovaries were encountered during the operation for removal.

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* Virchow's *Archiv*, cxxxiii, 165.

Another remote possibility presents itself, that, as in Kolaczek's case,* tissues from an ovarian dermoid might have been transplanted from the ovary to the peritoneal cavity and have grown there; but in such an event the original dermoid or teratoma would have been quite obvious in the affected ovary, while in the case under consideration the ovaries were expressly stated to have been found healthy.

According to Wilms,† very few instances of dermoid have been reported as occurring in the abdominal cavity proper, excluding those of the pelvis, most of which were single cysts, and judging from the cases reported in the literature instances of solid abdominal teratoma must be exceedingly rare. But Meckel, Bonfigli, Marchand and Maydl have each reported abdominal growths of a more complicated structure than simple dermoid cysts. Meckel's ‡ tumor was situated on the diaphragm and contained twenty-one pieces of bone, four teeth and some hair. Bonfigli's § case was that of a pear-shaped growth occurring in a strand of tissue between the liver and the stomach. It contained a piece of true bone in which were found two teeth, and nineteen more teeth lay free in the tumor cavity. The main cyst was lined partly with cutis. There were other cysts filled with mucus, but they were not further described.

One of the most complicated teratomas of the main abdominal cavity was described by Marchand.‖ It was situated behind the peritoneum, and on the aorta and left kidney, and contained a skull cavity, intestine, male genitals, prostate and cysts lined with ciliated epithelium. This case was therefore very far advanced developmentally, and was analogous to the following reported by K. Maydl¶: In a well developed but very anæmic man, 19 years of age, a tumor was found occupying the greater part of the pelvic cavity. Laparotomy was performed and the tumor was seen to lie between two folds of the root of the mesentery, and consisted of a well formed trunk and limbs. The head was absent, and in its place there was a tuft of hair 50 cm. long. The fœtus was enclosed in a firm amniotic sac that contained also some oily fluid. A thickening of the amniotic membrane, connected with the superior mesenteric artery, showed histologically the structure of the placenta. The umbilical cord was entirely wanting. The tumor was classed as a dermoid and was assumed to be a rudimentary twin, an instance of *foetus in foetu.*

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* Virchow's *Archiv*, lxxv, 39.

† Loc. cit.

‡ *Deutsch. Arch. f. Physiol.* (1815). Quoted by Wilms.

§ Ref. Schmidt's *Jahrb.* clxx (1876), 180. Quoted by Wilms.

‖ *Breslauer ärztl. Ztschr.*, 1881, No. 21. Quoted by Wilms.

¶ *Wiener klin. Rundschau*, x (1896), 295. Abstract in *Archives of Pediatrics*, December, 1896.

My case evidently is to be placed in the same category with the four cases just mentioned, and it is almost unnecessary to draw attention to the parallelism between the two last mentioned tumors, Marchand's and Maydl's, and the growth we are now considering. All three have made the impression on those examining them that they were dealing with tumors that were in reality independent individuals. All three were situated in the abdominal cavity; two of them, Marchand's and Maydl's, retroperitoneally, and one of them, the case in hand, intraperitoneally.

The age at which teratomata and dermoid cysts develop appears to vary much. Wilms, in his article previously referred to, speaking more particularly of dermoids of the ovary, makes the statement that they may appear at any time of life between intrauterine existence and the seventieth year, although the number occurring in childhood and old age is small, as the majority of cases occur during the second and third decade. In the case here reported the patient was twelve years of age. As the child had not yet begun to menstruate, no connection between a physiological increase of blood supply to the abdominal organs and the growth of the tumor could be definitely proven.

The relatively great development of the ectodermic structures (integument and nervous system) in the tumor is quite in accord with other knowledge, for we have evidence that both ontogenetically and phylogenetically the ectoderm takes precedence of both the endoderm and mesoderm. In speaking of dermoids of the ovary, Wilms says that "the advanced development of the ectoderm as well as the preponderance of the tissues of the head in these tumors are easily explained by the early differentiation, both of the epiblast and of the cephalic extremity in the fœtus." Wiedersheim,* in referring to the relative age of the ectoderm, states that the dermal skeleton is phylogenetically older than the endo-skeleton. Its relative age is shown not only by palæontology but also by ontogeny, *e. g.* the young file fish is provided with a complete dermal armor at the time when the ossification of the primordial cranium has hardly begun.

Our case is somewhat remarkable in that it is the third instance on record where the hair of a dermoid has been found growing free into the abdominal cavity. The first case is that reported by Kolaczek,† in which the patient, who was a spinster, 45 years of age, was operated on for a dermoid cyst of the ovary. At the operation a number of lentil-sized yellowish nodules were found scattered over the visceral and parietal peritoneum. Many of these nodules were pierced through their centre by a fine hair, and were regarded by Kolaczek as miliary metastases from the dermoid of the ovary. According to Wilms, Fraenkel ‡ has reported a similar case.

Until recently the existence of nervous tissues in dermoids and teratoma has been considered to be rare. Baumgarten,§ writing in 1887, made the statement that only six authenticated observations of the finding of central nervous tissues preceded that of his case. Recently, however, it has been shown that such tissue is really of extremely common occurrence, and one must believe that its supposed rarity in the earlier cases is due to the fact that it was not recognized. Wilms believes that tissues of the central nervous system are almost always, if indeed not constantly, present within ovarian dermoids, for he found it in 18 out of 19 consecutive cases which he examined. He believes furthermore that the finding of such tissue in a teratoma is a good argument for considering the particular tumor in which it is found as an independent individual.

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* Elements of the Comparative Anatomy of Vertebrates, p. 30. London, 1886.

† Loc cit.

‡ *Wien. med. Wochensch.*, 1883.

§ Virchow's *Archiv*, cvii, 515.

Wilms, in his investigations, frequently ran across corpora amylacea, and he states that they were of great value to him in locating the tissue of the central nervous system. In the very numerous sections of our tumor examined no corpora amylacea were found.

Eye structures have not been encountered very frequently, as up to the present I have been able to collect from the literature records of only fifteen cases where pigmented epithelium has been demonstrated in teratomata and dermoids. These cases have been reported by Marchand,* Baumgarten,† Lazarus,‡ Wilms § (3 cases), Kappeler,|| Pommer,¶ Rippmann,** Verneuil,** Kümmel,** Benno and Martin Schmidt,** O. Spöndly,** van Duyse,†† and Lovett and Councilman.‡‡ If the case in hand is added to the above mentioned we have a total of sixteen where pigment or pigmented epithelium has been found in teratomata.

The question of the recurrence of the growth and its malignancy has next to be considered. It has seemed to me worth while in this connection, on account of the great interest of this side of the subject, to review with some care the cases of teratoma which have been reported as recurrent or malignant.

The instances where teratomata have been found, either clinically or microscopically, to be malignant, or to have undergone malignant degeneration, are not numerous. This is somewhat surprising when one reflects that teratomata have been considered to be misplacements of fœtal tissue, and that true tumors, the malignant ones included, are also, according to Cohnheim's theory, supposed to originate from misplacements or implantations of fœtal tissue. The experiment of engrafting fœtal tissue into animals with a view to causing tumor formation has been tried fruitlessly, but, as Grawitz has said, teratomata fur-

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* Loc. cit.

† Loc. cit.

‡ Inaug.-Diss., Giessen, 1888.

§ Loc. cit. and Ziegler's *Beiträge*, xix.

|| Inaug.-Diss., Zurich, 1896.

¶ Abst. in *Centralbl. f. allg. Path. u. path. Anat.*, i (1890), 260.

** Cited by Kappeler, loc. cit.

†† Ref. Schmidt's *Jahrb.*, ccxlix (1896), 235.

‡‡ *Journal of Experimental Medicine*, ii (1897), 427.

nish an example of the implantation of fœtal tissue better than any artificial implantation could, for in teratomata the implantation of fœtal tissue occurs in fœtal tissue, while artificially the grafting must be done at least on post-gestational tissue. The instances I have collected in which teratomas have been malignant, or have undergone malignant change, are as follows:

I.—Czerny's case.* A congenital sacral tumor, consisting for the most part of fat, and containing cysts lined in some instances with ciliated, in others with flat, epithelium. The patient was 55 years of age, and the tumor underwent malignant change after repeated traumatisms. Czerny demonstrated the metaplasia of ciliated into flat epithelium, with pearl formation. After extirpation there was local recurrence, followed by metastasis of flat epithelial cancer in the inguinal glands.

II.—Virchow's case.† Teratoma myomatodes mediastini. This was a large, lobulated, and, for the most part, smooth tumor occupying the right side of the chest of a man, 22 years of age. The tumor contained striated muscle tissue, sarcomatous tissue, and masses of hyaline cartilage, surrounded by well marked perichondrium. There was a considerable number of cysts in the tumor, some of which resembled proliferating ovarian cysts. Other cysts were lined with skin, others with columnar ciliated epithelium, and still others with simple columnar epithelium. In the middle, more compact mass of the tumor there was tissue having the structure of carcinoma. A metastatic nodule in the left third rib was cystic (simple cysts), sarcomatous and carcinomatous, and contained striated muscle fibres, and a portion of this metastasis recalled vividly the structure of fœtal lung. The structure of nodules in the liver and kidneys was not determined.

III.—Jores' case.‡ Combination of an intrathoracic dermoid cyst with malignant cystosarcoma of the left lung. There was nothing of striking interest in the dermoid cyst itself. Its walls, however, were cystic, and these cysts evidently arose from the sebaceous and sweat glands of the dermoid. The intercystic stroma contained many islands of hyaline cartilage, much smooth muscle fibre, and much spindle-celled tissue. Besides the above mentioned cysts there were others more irregular in shape and lined with cylindrical epithelium. This cylindrical epithelium did not sit on a basement membrane, but sat directly on the spindle-celled tissue. The metastatic nodules in the right lung had exactly the same structure as the main tumor.

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* *Arch. f. klin. Chir.*, x, 894.

† Virchow's *Archiv*, liii, 444.

‡ Virchow's *Archiv*, cxxxiii, 66.

IV.—Virchow and Litten's case.* A case of androgyny with malignant teratoid cystoma of the right ovary and bilateral cystic hydrocele of the processus vaginalis of the peritoneum. A multilocular cystic tumor, apparently of the right ovary, developed in a female, 16 years of age. Here and there in the tumor were small dermoid cysts. Medullated nerve fibres, smooth muscle fibres, and masses of hyaline cartilage provided with perichondrium were present in the stroma. In some places the tumor was sarcomatous (round-celled) and in others myxomatous.

In the metastatic nodules in the liver there was neither epidermis nor hair nor cartilage. There were small and large cysts, like ovarian cysts, and the stroma corresponded to the sarcomatous and myxomatous portions of the original tumor. No carcinomatous structure was seen.

V.—Wernitz's case.† A teratoma of the right ovary occurring in a woman, 25 years of age. The tumor had a very complicated structure. The main part of it was formed of small colloid and dermoid cysts. Some of the cysts were lined with simple epithelium, others with columnar epithelium. The dermoid cysts were lined with stratified horny epithelium, and provided with hair and sebaceous and sweat glands. In many places in the connective tissue stroma there were hyaline cartilage and small lamellæ of bone. In other places there was large-celled sarcomatous tissue, and here and there were groups of epithelial nests.

At the autopsy metastatic nodules were found in the peritoneum, lungs, kidneys and liver. These metastatic nodules consisted of richly cellular sarcomatous tissue that resembled closely that of the primary tumor.

VI.—Keller and Kramer's case.‡ The patient was a multipara, 20 years of age, who suffered from a quickly growing, nodular tumor of the right ovary. There was much ascites. The tumor was surrounded by a hard fibrous capsule, and scattered throughout it were many small islands of cartilage and pieces of bone. There were some cysts, one the size of a fist, but the tumor was for the most part solid. Microscopically there were sarcomatous tissue, fat, cartilage and bone. Some of the cysts were lined with stratified epithelium, and others with stratified epithelium that gradually changed into cylindrical epithelium. These cysts were provided with sweat and sebaceous glands and hair. Other cysts were lined entirely with simple or stratified epithelium and had no sweat glands, etc. Smooth muscle fibre was present, but no striated muscle fibre and no nerve tissue.

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* Virchow's *Archiv*, lxxv, 329.

† *Ztschr. f. Geburtsh. u. Gynak.*, xxxi, 417.

‡ *Ztschr. f. Geburtsh. u. Gynak.*, xii, 170, and xviii, 124.

After the operation there was a quick return of the ascites and a recurrence of the tumor.

The patient died eleven months after the operation, and the post-mortem examination showed an extensive return of the tumor in the peritoneum and metastases in both lungs. Microscopical examination showed the secondary tumors to be sarcomatous, and in one of them, situated in Douglas' sac, islands of cartilage were found.

VII.—Emanuel's case.* A girl, 15 years of age, was operated upon, January 30, 1892, for a tumor of the left ovary, the size of a man's head. A quantity of brown ascitic fluid was found in the peritoneal cavity. There were a great number of small cysts scattered throughout the tumor, some lined with skin provided with hairs and sebaceous glands, others had no lining membrane, others were clothed with stratified cylindrical epithelium, and others with simple cylindrical epithelium. Some cysts were lined with cylindrical epithelium that gradually changed to cuboidal or flat epithelium. One cyst was lined with ciliated stratified epithelium. The mass of the tumor consisted of round-celled sarcomatous tissue divided off into fields by strands of connective tissue. There were also smooth muscle cells, small oval or round masses of hyaline cartilage provided with perichondrium, fat, mucoid tissue, and black and golden-yellow pigment partly intra- and partly intercellular.

The recurrence was speedy, for in five weeks there was a demonstrable ascites, and in a fortnight more there were unequivocal symptoms of the presence of an abdominal tumor. The patient died, June 2, 1892, four months after the operation.

There was a recurrence *in loco*, and the right ovary, that at the time of operation seemed to be perfectly healthy, was now changed into a tumor the size of two fists. There were metastatic tumors in the subcutaneous cellular tissue of the abdomen, on the parietal and visceral peritoneum, on the under surface of the diaphragm, in both omenta, the suspensory ligament of the liver, and on the lower surface of the liver. Microscopically, the recurrence *in loco* and the tumors of the omenta were identical with the original growth. The tumor of the right ovary was also similar, but with the addition of blood cysts. The tumors of the peritoneum resembled the sarcomatous stroma of the original neoplasm. The tumor lying in the subcutaneous cellular tissue of the skin of the abdomen was a surprising structure, and is of the utmost interest to us, as it was not simply a sarcoma, but a compound tumor like the original neoplasm of the left ovary. It had a complete connective tissue capsule. The stroma of the tumor was, as in the original, composed of round and spindle-celled sarcomatous tissue, and there were numerous cysts, lined with various kinds of epithelium, some with simple or stratified columnar, others with flat or cuboidal epithelium. As in the original tumor, there were solid columns of flat epithelial cells, and some of the cysts lined with flat epithelium did not have a basement membrane, while all the other cysts had. Besides these structures small pieces of cartilage and sections of glands were found.

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* *Ztschr. f. Geburtsh. u. Gynäk.*, xxv, 187.

VIII.—Lazarus' case.* A tumor weighing 9000 grammes was removed from the right ovary of a woman, 27 years of age. It was decidedly lobulated; the centre of each lobe was formed of cartilage, and around the cartilage the tissue was honeycombed with small cysts.

The following tissues were found on microscopical examination: Hyaline cartilage merging into fibro-cartilage and mucoid tissue; connective tissue, some of which was sarcomatous; adipose tissue; myxomatous tissue with branched cells; elastic tissue; flat epithelium with cholesteatoma pearls; cysts with distinct flat epithelium; cysts formed from dilated glands; and cysts lined with skin provided with sweat glands, hair follicles, but without hair or sebaceous glands. In addition to these structures there were smooth and striated muscle fibres; glands with simple cylindrical epithelium; typical goblet cells like those of the intestine; cysts with ciliated epithelium; neuroglia; varicose medullated nerve fibres; ganglion cells like those of the brain and sympathetic system; and finally eye-like structures. These last lay in the neighborhood of tissue resembling gray brain-matter.

No hair nor sebaceous glands nor bones nor teeth were found. The author, however, believed he discovered indications of fœtal tooth formation.

The tumor grew with immense rapidity, and other tumors were found in Douglas' sac and on the diaphragm which were thought to be implantation growths. The growths in Douglas' sac contained cartilage, and cysts lined with goblet and cylindrical epithelium; those on the diaphragm were made up entirely of mucous tissue.

IX.—Biermann's case.† This was a spindle-celled sarcoma of the ovary, the size of a man's head, and contained a dermoid cyst the size of an apple.

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* Ein grosses Teratom des Ovarium. Inaug.-Diss., Giessen, 1888. Abstract by Wilms in Ziegler's *Beiträge*, xix, 369.

† *Prag. med. Wochensch.*, 1885, No. 21.

X.—Ewald's case.* From a girl, 21 years of age, a teratoma of the ovary the size of a man's head was removed by laparotomy. Microscopical examination did not reveal any carcinomatous or sarcomatous tissue. Three weeks later there was a recurrence as large as the first tumor; this was removed, and at the same time the opposite ovary was excised, as it was also the seat of teratoid disease. A further recurrence was demonstrable ten days after the second operation, and two weeks later the recurrent tumor had attained to the size of a child's head. After several weeks a third operation was done, but the patient died. The autopsy showed that the tumor in the last instance had arisen, not from the region of the ovary, but from the omentum; the peritoneum was studded over with nodules the size of a pea. In addition, behind the peritoneum and in the mesocolon there were two tumors larger than pigeon's eggs. Neither sarcoma nor carcinoma could be demonstrated anywhere. The nodules in the peritoneum consisted exclusively of embryonic connective-tissue cells. Ewald draws the conclusion that teratoma of the ovary is in itself a malignant tumor, and that metastases can occur without carcinomatous or sarcomatous degeneration and without alteration of the histological structure.

I have not included in the foregoing list cases without recurrence or metastasis where it is stated simply that part of the stroma of the teratoma presented a sarcomatous character or nests of epithelium.

There are still some instances where teratoid tumors have undergone malignant degeneration, but they differ so markedly from the foregoing that they require separate mention. A few cases have been observed where flat-celled epithelioma has sprung from the skin lining the internal surface of what have always until lately been looked upon as simple dermoid cysts of the ovary. It would seem, however, that these presumably simple dermoid cysts are in reality complex tumors, for Wilms found in every case examined by him tissues representing all three embryonic layers. If all the ovarian dermoid cysts taken at random and examined by Wilms were compound tumors, one can justly assume with him that this is always their nature. But if all ovarian dermoid cysts are compound tumors, then all instances of malignant degeneration in such cysts would be instances of malignant degeneration in compound tumors. Although Wilms has found these dermoid cysts to be uniformly so complex, yet he does not believe that these ordinary dermoid cysts of the ovary should be classified with teratomata occurring in other parts of the body, and would consider them entirely apart and by themselves.

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* *Wien. klin. Wochenschr.*, 1897, No. 10, p. 226. Abstract in *Centralbl. f. allg. Path. u. path. Anat.* ix (1898), 177.

Epitheliomatous malignant degeneration has been reported as occurring in ovarian dermoid cysts by Heschl,* Biermann,† von Wahl,‡ Pilliet,§ Tauffer,|| Himmelfarb,¶ Krukenberg,** Veit,†† Wilms,‡‡ Klein,§§ Yamagiva |||| (2 cases), and Thumim,¶¶ thirteen cases in all.

In speaking of carcinomatous degeneration of ovarian dermoids, Thumim says that in looking over the literature he considers that the cases of Heschl, v. Wahl, Biermann, Himmelfarb, Krukenberg, Tauffer, and the second case reported by Yamagiva are the only undoubted examples of this affection which he has been able to find. Nine other cases that have been reported he cannot accept, partly on account of uncertainty that the disease was primary in the ovary, and partly on account of insufficient microscopical investigation.

In considering whether the adeno-carcinoma-like structures described in the case here reported were really cancerous or not, one must bear in mind that what appears to be identity of structure does not necessarily indicate identity of nature, and that structurally a resemblance to cancer may be simulated when one does not really exist. It does seem, however, that the adenomatous tissue here was malignant and therefore constituted an adeno-carcinoma. The epithelium was thrown about in a most disorderly fashion in alveoli having no basement membrane. Furthermore, these structures were heterolo-

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* *Prager Vierteljahrsschr.*, lxviii (1860).

† *Prager med. Wochenschr.*, 1885, No. 21.

‡ *Petersburger med. Wochenschr.*, 1883, p. 70.

§ Cited by Wilms, *Deutsch. Arch. f. klin. Med.*, lv, 373.

|| Virchow's *Archiv*, cxlii, 389.

¶ *Centralbl. f. Gynäk.*, 1886, 569.

** *Arch. f. Gynäk.*, xxx, 245.

†† *Centralbl. f. Gynäk.*, 1890, 283.

‡‡ Loc. cit.

§§ Inaug.-Diss., Freiburg, 1893.

|||| Virchow's *Archiv*, cxlvii, 99.

¶¶ *Arch. f. Gynäk.*, liii, 547.

gous, for, normally, glands of this or any other character do not occur scattered at random throughout a living being, and heterology is an important element in the estimation of malignancy. The whole tumor was structurally embryonic in character, and looked as if rapidly growing, and clinically it was a rapid grower and recurrent; and embryonic structure, rapid growth and recurrence are of more weight even than heterology in arriving at a diagnosis of malignancy, but here all these combined in pointing to a malignant nature. Nevertheless it can be objected that metastasis was not proven, that infiltration of neighboring tissue was not a marked feature, and that the embryonic character and rapid growth of this glandular epithelium might simply have been co-equal with the embryonic character and rapid growth of the rest of this parasitic individual.

If, however, it be granted that the adeno-carcinoma-like structures in this case were really adeno-carcinomatous, then it is a very rare event indeed, so rare that Tauffer says no case of adeno-carcinomatous degeneration in a dermoid has ever yet been described.* Some of the structures in Jores' case, mentioned above, seem, however, to have struck the author as resembling adeno-carcinoma, for he describes some of the cysts as being irregular in shape and lined with cylindrical epithelium which did not rest on a basement membrane, but stood directly on the spindle-celled tissue of the stroma of the tumor. Metastatic nodules in the right lung were said to be exactly like the main tumor, and therefore, presumably, had also the above mentioned irregular cysts lined with columnar epithelium. In Emanuel's and in Lazarus' cases, already quoted, there were cysts in the metastases lined in the former with stratified epithelium and in the latter with goblet cells and cylindrical epithelium.

Some might assert their disbelief in the tissue removed at the second operation being a recurrent growth, principally because of the immense variety of structures found in it. They might say, that besides the three obvious lobes removed at the first operation there were probably other original implantation tumors scattered over the peritoneal surface that had not yet attained such a size as to attract attention, and that the tissue removed at the second operation was the tissue of original implantation tumors that had grown into prominence in the intervening time.

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* Op. cit., p. 391.

Against this opinion is to be urged what Dr. McNutt told me: that at the first operation the peritoneal cavity was, as far as he could see, perfectly healthy and free from tumor growth, excepting the area from which the original tumor grew; also that the large mass of the tissue removed at the second operation was taken from the site of the original tumor; that at the post-mortem the spread of the tumor had taken place as a continuous layer over the peritoneum; and that there were no indications at any point that this continuous layer of new tissue had been formed by the coalescence of smaller tumors.

The term "recurrent tumor" has been continually made use of throughout this article to designate the tissue removed at the second operation. By "recurrent," however, is meant that some particles of the original tumor, too small to be observed by the operator, were left behind and had grown rapidly so as to form the large tumor met with at the second operation. The probability that some of the original tumor was left unremoved at the first operation was shown by the discovery of the small piece of bone in the tissue extirpated at the second operation, for the time between these two operations seemed too short for fully developed bone to form.

A very small portion of the original tumor left behind and growing again would be sufficient to account for the great variety of tissues found in the recurrent tumor. To illustrate this more fully it is only necessary to relate that, at the suggestion of Dr. W. G. Hay, transverse sections were made of a bundle of the strings connecting the lobes of the original tumor. The diameter of none of the strings was greater than that of ordinary twine, but it was surprising to see the variety of structures, both epithelial and connective, encountered in each of them.

The incomplete removal of diseased tissue and the rapid reappearance of a tumor at the point of operation is, it is needless to say, a frequent and often unavoidable occurrence, and constitutes an important factor in the group of events to which the term malignant is given. Malignancy in the present instance consisted of rapid growth, recurrence and the property of spreading by continuity. The tumor did not appear to have given rise to metastases, although from its structure this kind of malignancy might have been expected, and has been observed several times in similar teratomata.

Some of the different theories that have been framed in the past to account for the origin of teratomata may now be considered, and an opinion arrived at as to which one of them best fits the conditions found in the present instance.

I.—Many dermoids and teratomata are accounted for by a dipping down or involution of the external skin and subsequent pinching off. In such cases a cyst is formed with the epithelium of the involuted skin lining its inner surface. This theory does not, however, explain the present case, which was a solid teratoma covered by skin.

II.—Many teratomata admit of the explanation that they arise from a simple misplacement of a group of cells during development. The free situation in the abdominal cavity, the integumentary covering, and the great variety of structures in the case in hand would, however, all be against this view of its origin.

III.—Some authors have thought that irritation of a tissue may cause a compound tumor consisting of many varieties of the tissue irritated. This theory may be dismissed without comment.

IV.—What is called partial double formation sometimes takes place, that is to say, the same generative act that is responsible for the main being or individual also gives rise to a double formation of some of its parts. This theory satisfactorily explains the formation of supernumerary limbs, but hardly accounts for a tumor in which such diverse organs as skin, gut, nervous system and eye were found.

V.—At times evidently a complete double or twin formation takes place, with partial growing together or fusion of the twins. Many of the double monsters would appear to originate in this way, but the case in question is not to be so explained, for although there were two beings, no fusion had taken place between them. One individual simply grew inside the other.

VI.—It has been presumed that the unfecundated ovum can proceed to develop without the assistance of the spermatozoon, and form an imperfect individual. The spermatozoon also without the concurrence of the egg is thought by some to have some formative power. This so-called parthenogenesis is supported by numerous examples in the lower orders of life, where the fecundation of the female is only required every few generations.

This possibility requires to be carefully weighed in the present case, as the egg is the only cell that we know of that can give rise to an individual having all three embryonal layers. And the converse is also true, that an individual having all three embryonal layers must arise from an egg. Therefore the individual under consideration necessarily originated from an egg. If this hypothetical egg came from the ovary of the child in whom the tumor was found, then this would be a case of parthenogenesis. This child, however, had not menstruated, and therefore presumably had not ovulated. It must be admitted, however, that it is thought that an egg may go on to development in the ovary before it has ovulated. But if this had been the case in the present instance the dermoid would have been found in the ovary, or would at least have been attached to it by a pedicle. As before stated the ovary was normal, and there were no traces at all of a pedicle or of a union between the tumor and the ovary.

VII.—It is held that two individuals may begin to develop from their respective ova, but before development is far advanced one may be enclosed by the other, and the included one's growth be so interfered with that it does not continue to develop but lies dormant for an indefinite time. This is called *foetus in foetu,* and is the most natural explanation of the facts in the present instance. It is easy of comprehension to suppose that during this little patient's fœtal life, and before the abdominal cavity closed, another egg, and possibly a fecundated one, slipped into the open peritoneal sac. The egg lay there as in a pocket up to the child's twelfth year, when it began to grow, but in a disorderly fashion and with all the energy of fœtal life. In this view the tumor would represent the patient's twin brother or sister.

NOTE ON DR. D. W. MONTGOMERY'S CASE OF TERATOMA.

By Lewellys F. Barker.

Through the courtesy of Dr. Montgomery I have been permitted to study sections from his tumor and to make (1) a note upon the nervous tissue present, and (2) some remarks concerning the theories which have been advanced to explain teratomata in general.

I.

The tumor contains large masses of nervous tissue distributed throughout its substance. While most of this nerve tissue resembles embryonic brain, including the lining of the central cavity with chorioid plexus, some medullated nerve fibres were discovered and also traces of embryonic eye structures. The masses of brain substance are scattered very irregularly through the tumor and at first sight apparently lawlessly. The size of the individual masses varies much, from small islands of the tissue recognizable only with the aid of the microscope to relatively large areas easily visible to the naked eye. Some of the masses are bounded definitely on one side by a cavity lined by cubical epithelium (rudiment of ventricles and ependymal epithelium), and on another side by vascular connective tissue (brain-membranes?). Other portions of brain substance are found for which no ventricular nor meningeal relations can be made out. In places the lining of the cavities supposed to represent the ventricles is seen to be made up of low, clear, cubical epithelium, which can be followed as a definite *lamina chorioidea epithelialis* over the much branched and anastomosing papillary strands of vascular connective tissue which project into the cavity, forming a distinct *plexus chorioideus* (Plate XXII, Fig. 13). No cilia could be made out, however, on any of the cells lining the cavities. In certain regions, instead of a lining with the characters just described, the tissue adjacent to the cavity resembles more closely that seen in certain regions in the embryo, for example that of the developing corpus striatum and of the developing spinal cord.

The ependymal epithelium is divisible into three distinct zones—an inner, middle, and external zone. In the middle zone of each cell is placed the oval nucleus, which stains in hæmatoxylin, the nuclei of adjacent cells being placed at different levels, so as at first sight to give the impression of a mass of cells several layers deep. The internal and external zones of these cells are protoplasmic and stain intensely with eosin. The inner zone is narrow, and the outer zone, corresponding to the *Randschleier* of His and to the embryonic white matter, is much wider.

No cells which resembled adult nerve cells could be made out. Among the nuclei in the nerve tissue there were many small round rather solid nuclei resembling those of neuroglia cells; other nuclei were larger, only slightly oval and more vesicular. Some of the latter may be nuclei of neuroblasts, but the preservation of the tissue has not been such as to permit of a positive statement with regard to this point. Besides, nowhere in the masses of brain substance was it possible to make out definitely any axis cylinder processes of nerve cells. By far the most of what looks like brain tissue consists of a pale reticulum-like mass of fibrils (glia fibrils and processes of ependymal cells?), in the meshes of which are many small round cells with compact chromatin and scanty protoplasm (glia cells?).

Here and there embedded in the mass of pale glia-like tissue is to be seen a compact nodule, consisting of closely crowded small cells with scanty protoplasm and solid round nuclei. These nodules are not separated by any membrane from the surrounding tissue (Plate XXII, Fig. 12). What they represent it is difficult to say. One could think of masses of embryonic basal ganglia or simply of enormous aggregations of glia cells.

No traces of spinal ganglia such as Kappeler has described in one of his cases could be found. The only medullated nerve fibres observed were in the sections of skin. In these, several bundles of typical adult fibres with myelin sheath and neurilemma were made out.

As regards the presence of sense organs the tumor is notable in that it contains distinct evidence of rudimentary eye structures. Microscopic examination of the pigmented sheet of tissue found in the gross study of the tumor attached by one edge to a bone showed it to be derived from an incomplete eye. Dr. Montgomery states that one surface was covered by a layer of flat, nucleated polygonal cells. The protoplasm of these cells is closely studded with fine brown, rod-shaped, pigmented granules (Plate XXII, Fig. 11). The cells are arranged in the form of a mosaic, and correspond to the stratum pigmenti retinæ derived from the portion of the ophthalmic vesicle not invaginated in the formation of the optic cup. No other part of the body resembles this structure. The layer of pigmented cells rests upon a very firm connective tissue membrane, which contains a few slightly pigmented connective tissue cells. No definite tunica vasculosa (chorioidea) could be made out, and the tough fibrous tunic adjacent to it, in places distinctly lamellated, probably corresponds to the sclera, though it would be difficult to prove this positively. In the original tumor no trace of the nervous portion of the retina nor of the optic nerve was discovered. In addition to the stratum pigmenti described, similar polygonal pigmented cells are to be seen irregularly distributed through the sections, sometimes apparently in solid portions of the tissues, sometimes lining small irregular slit-like spaces, and in one instance lining a space which is continuous with the cavity lined by ependymal epithelium, which probably corresponds to brain-ventricle. The latter observation is all the more interesting in that, as is well known, during development the cavity of the ophthalmic vesicle is continuous with the cavity of the central nervous system; the vesicle on each side arises as an outgrowth from the first cerebral vesicle at a point corresponding to the junction of the diencephalon with the telencephalon, marked even in the adult by a distinct pocket, the so-called recessus opticus. The pigment in all the cells mentioned was absolutely typical and unmistakable. There was no possibility of confounding it with hæmatoidin or with other pigments of hæmic origin.

The nerve tissues could not be studied by Golgi's method, as they had been hardened in alcohol. An attempt to impregnate pieces with bichromate subsequently proved unsuccessful.

If ear, nose and tongue were represented in the tumor, they either did not happen to be struck in the sections made, or, as is not unlikely, the tissues corresponding to them were too atypical to be recognized.

Masses of nerve tissue of the same general type as described above made up a large part of the recurrent tumor. Numerous areas were found in this in which there were traces of stratum pigmenti retinæ. The lining of some cysts consisted partially of such a pigmented layer.

The existence of peripheral nerves along with tissues of the central system appears to be rare in teratomata, though instances have been described by Baumgarten, Kappeler and others. It is interesting to consider the origin of the peripheral nerve fibres. In the cases in which well developed ganglion cells are present in the tumor one could think of the axones of the peripheral nerve fibres as direct processes of these cells, especially in cases such as Kappeler's, where well formed spinal ganglion cells were made out and photographed. In Dr. Montgomery's case no ganglion cells, at least none approaching in appearance the adult forms, could be found within the tumor. One could explain the origin of the medullated fibres in either of two ways: (1) by assuming the existence of ganglion cells in the new growth which were by accident missed in the microscopic study, or (2) by assuming that the nerve fibres represent ingrowths from the body of the patient herself, when the axones would represent prolongations of some of the nerve cells of the host. The latter view, if it be the correct one, need not necessarily be surprising, considering the recent findings of Young [*] concerning the presence of nerves in sarcomata and other tumors.

In view of the fact that masses of central nervous system, spinal ganglia and peripheral nerve fibres may be present in teratomata, it need not excite wonder that the organs of special sense are sometimes represented. Indeed it would be surprising were they absent.

II.

Many theories regarding the origin of teratomata have been brought forward. Among the best general discussions of the subjects are those of Meckel [†] and of Klebs.[‡] The theory which suggests that such a tumor as the one described in the present article can result from the irritation of tissue cells in the region in which it arises may be dismissed without further consideration. An ovarian or abdominal pregnancy is also out of the question, for besides the fact that no placenta existed, the child was *virgo intacta* and had not even menstruated. Moreover, teratomata may appear at any time of life, and in males as well as in females.

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[*] *The Journal of Experimental Medicine*, ii (1897), 1.

[†] Meckel, J. F., Handbuch der pathologischen Anatomie, 1812-1818.

[‡] Klebs, E., Die allgemeine Pathologie, Theil ii, Jena, 1889, p. 797.

The doctrine of parthenogenesis and its various modifications, while supported by many able investigators in the past, is at present held by scarcely any one and need not therefore be discussed.

The theories which make the origin of teratomata congenital have most in their favor. But that in this case we have to deal with a tumor which has arisen from a dipping down or involution of the external skin with subsequent pinching off, as has been assumed for many teratomata, is clearly impossible. The variety of tissues present is too great, and the fact that we had to do, not with a cyst lined by skin on its inner surface, but with a solid teratoma covered by skin on the outside, excludes any such simple explanation.

One of the oldest of the modern theories regarding the origin of teratomata is that of Meckel, the theory of *foetus in foetu.* This theory, which makes the teratoma and its host twin individuals, accords best with the facts in the majority of instances and especially with those of the present case.

Klebs has divided the teratomata into (*a*) the *endogenous* forms, which represent tumors which have resulted from the pinching off of tissues during development, and (*b*) the *ectogenous* forms, which he believes to be always the result of fœtal implantation. As he says, however, the limits between the two must remain doubtful, and it is not impossible that many teratomata which have been included among the endogenous forms are in reality ectogenous teratomata which have undergone marked involution. The case before us would naturally belong to the ectogenous group of Klebs. His idea that in such cases we have always to do with two more or less separate fœtuses, one of which grows around and partly or wholly includes the other, seems a very probable one. He distinguishes two forms of ectogenous teratomata: (*a*) the allantoid variety, in which one fœtus is situated in one of the body cavities of the other, and its blood-vessels enter into communication with those of the including fœtus, and (*b*) the pre-allantoid variety, in which there is a direct fusion, complete or partial, of the disc-like germinal areas of the two fœtuses at a very early stage, one finally gaining the ascendancy and growing around the other so as to include it. In the allantoid forms there is always an umbilical cord and sometimes a placenta; while in the pre-allantoid forms the umbilical cord and placenta are absent, and the included fœtus often shows very marked alterations and irregular development, probably corresponding to disturbances of vascular supply. In such instances the development may be so irregular and atypical that only microscopic examination reveals the true nature of the growth.

If we accept Klebs' hypothesis, then the case before us would agree best with the last mentioned group, and would therefore have to be considered as an instance of the abdominal form of the pre-allantoid ectogenous teratoma.

It is interesting to study the many transitional forms which are to be found between completely separated twin-formations and these complex teratomata * which may represent twins, one of the twins having included its fellow inside its own body. One has only to consult the atlases of Ahlfeld, Förster, Hirt and Piersol, Taruffi and others, to convince himself of large numbers and varieties of transitional forms. Thus instances in which one fœtus is partly inside and partly outside the body of a second are not at all uncommon. The well known examples of parasitic thoracopagi, of engastrius, epignathi, encranius, notomelus, pygomelus, etc., will be immediately recalled. Then there are forms in which one fœtus is entirely enclosed within the body of the second. It may be beneath the skin (inclusio subcutanea), it may be in the mediastinum, the abdominal cavity (inclusio fœtalis abdominalis), the sex glands, or even inside the skull. They are nearly always in situations in which embryonic inclusion can be easily accounted for. Some of these included forms are easily recognizable by the naked eye as independent individuals, but many of

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* Marchand, in a masterly review of the whole subject of malformations, published in Eulenburg's Real-Encyclopädie der gesammten Heilkunde (3rd edition), although he places dermoids and teratomata in a group by themselves, states that he can make no fundamental distinction between them and genuine fœtal inclusions. He assumes, therefore, that both have a similar origin.

them are remarkably transformed and difficult to identify. They are frequently acardiac monsters, the mode in which the blood vascular relations in the one fœtus have become dominant at the expense of the heart and blood-vessels of the second having been well shown by Thoma in his Text-Book of General Pathology.*

It is usually assumed that in fœtal inclusions we have to deal with two eggs, separately fertilized. As has been suggested by Sobotta, Marchand and others, it is not impossible that one or more "directive corpuscles" become fertilized and give rise to an embryo, since it has been proven by Mark, Bütschli and Boveri that they represent abortive eggs, and Platner has actually seen such structures penetrated by spermatozoa.

That the included embryo should remain dormant (in the present case for twelve years, in other instances in the bibliography much longer), and after the lapse of a long time give rise to serious symptoms, is difficult to explain satisfactorily, but it must be remembered that as yet our knowledge of the conditions underlying the mechanics of development is extremely meagre. We have evidence in comparative embryology which permits the statement that a fertilized egg may be placed under conditions which preclude cell-division, and that it may later under more favorable circumstances go on to normal development.

The question as to the possible origin of such a tumor as a consequence of splitting in the long axis of a single embryo at a very early stage must not be overlooked. In view of the results of modern experimental teratology such an origin cannot be excluded. Certainly many of the cases of anadidymi, katadidymi and the like are best explained by assuming a partial splitting at an early stage. The objection that formation of double parts or extra organs, or even an entire second embryo, would necessarily lead to gross defects in the development of the original embryo, no longer holds, since Driesch, Wilson and Morgan have demonstrated the remarkable regenerative power of the very young embryo after injury (Roux's post-generation). We know now that in many animals, after fertilization and segmentation, a whole embryo may develop from each of two segmentation-cells iso-

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* English translation by Alexander Bruce, London, 1896, vol. i, p. 183.

lated by shaking. Even in the eight-cell or sixteen-cell stage, small but complete larvæ may develop from a single cell if it be isolated from its fellows. Of more than ordinary interest is the observation of Roux who noticed such isolated segmentation cells inside the bodies of young frog larvæ and suggested that they might be of significance in the etiology of tumors. The curious admixture of tissues, the marked excess of certain elements and the deficient development of other parts in teratomata need not surprise us when we consider the extraordinary disturbances to which the developing tissues in many of these cases are exposed.

No matter what view we take concerning the mechanisms of development and tissue-differentiation, be it Roux's modification of the evolutionary conception of Weissmann, or the more epigenetic doctrine of Hertwig, or a compromise between the two as suggested by Benda, there can be but little doubt that in the most complicated teratoma, where the arrangement of tissues is apparently most lawless, there is in reality no condition present which dare be considered as accidental. The relations which exist are the result of the response of activities inherent in the included embryo (and possibly activities which in the beginning were entirely normal) to the extraordinary influences from the environment to which such an embryo is subjected, and of the consequent manifold alterations of correlations inside it.

Concerning the nature of the primary causes which can lead to such a splitting in one embryo, or to the establishment of relations between twin-embryos which are destined to be disastrous to one and sometimes (perhaps only after years have passed) to both of them, we are as yet almost completely ignorant. The experiments of Newport, Panum, Valentine, Dareste, Gerlach, Pflüger, Roux, Born, Loeb, Hertwig, Ballantyne, Morgan, E. B. Wilson and others have in the field of comparative teratology demonstrated the effects of alterations of temperature and position, or of chemical relations such as modification of the oxygen supply, or the introduction of toxic substances, the influence of polyspermia, of pressure, shaking, and operative insult upon fertilized eggs and upon embryos in very young stages. While we dare not transfer directly the results of these experiments to the domain of human teratology, still the mass of data which they have afforded concerning the possibilities of developing cells may, along with further experiments in the same field and careful studies of pathological human embryos, open up the way which will lead ultimately to the construction of a satisfactory hypothesis concerning the nature and mode of origin of malformations and teratomata in human beings.

That teratomata, though undoubtedly of early embryonic origin, may appear at almost any age in life; that many show inside them tissues far removed from the normal type, sometimes one form of tissue predominating, sometimes another; further, that the mode of their growth and termination varies exceedingly; and finally, that metastases undoubtedly occur in certain instances—all these facts taken together lend support, it must be granted, in a very striking way to the doctrine of Cohnheim regarding the origin of tumors in general.

DESCRIPTION OF PLATES XIX–XXII.

PLATE XIX.

Fig. 1. Photograph of the original tumor.

Fig. 2. Skin of the original tumor.

PLATE XX.

Fig. 3. Part of cyst-wall showing goblet cells. Original tumor.

Fig. 4. A tube lined with cuboidal epithelium and surrounded by spindle-celled tissue in which islands of cartilage lie scattered. From the original tumor.

Fig. 5. Showing hair follicle from the original tumor. The first layer of cells lying on the basement membrane of the hair follicle have their nuclei far removed from the base of the cells.

Fig. 6. Bed hair from the original tumor.

Fig. 7. A typical epithelial tissue resembling carcinomatous infiltration.

PLATE XXI.

Fig. 8. Embryonic intestine provided with developing villi and surrounded by smooth muscle. Original tumor.

Fig. 9. Metaplasia of columnar epithelium into flat-celled epithelium with pearl formation. Recurrent growth.

Fig. 10. Pearl formation in recurrent growth.

PLATE XXII.

Fig. 11. Pigmented polygonal epithelium resembling that of the retina. Original tumor.

Fig. 12. Section showing embryonic nervous tissues. Recurrent growth.

Fig. 13. Section showing embryonic nervous tissue (glia) (C. n. s.) and cavity lined with ependymal epithelium and choroid plexus (Pl. ch.).

The drawings, with the exception of Figs. 8 and 13, were made by I. Katsuki, M. D., Univ. of California.

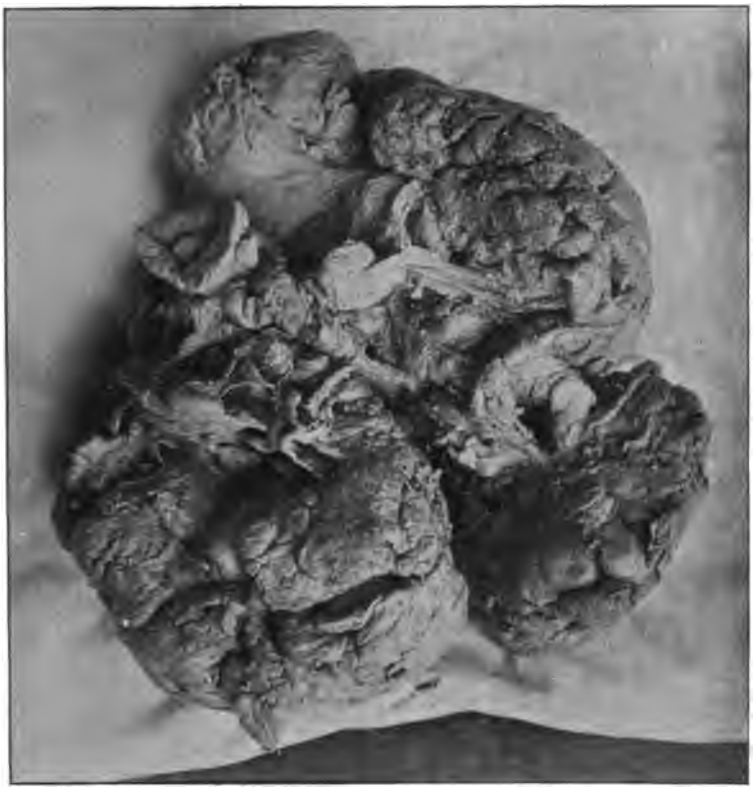


FIG. 1.

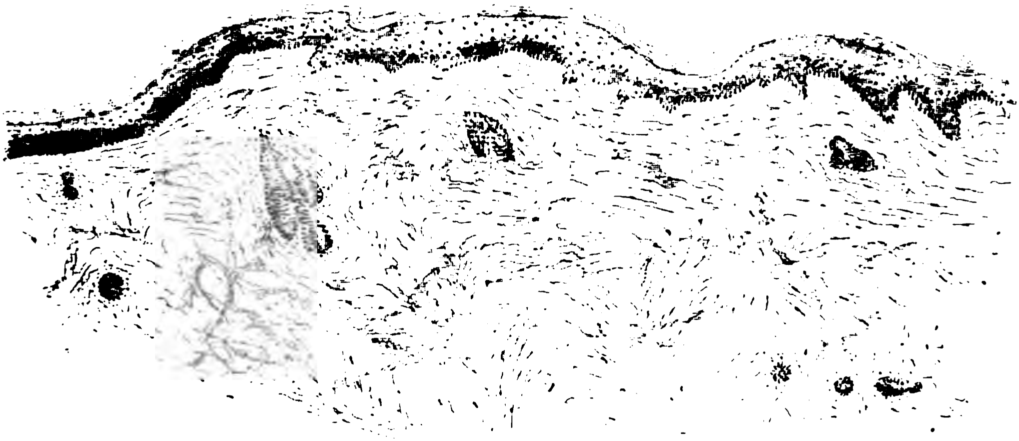


FIG. 2.

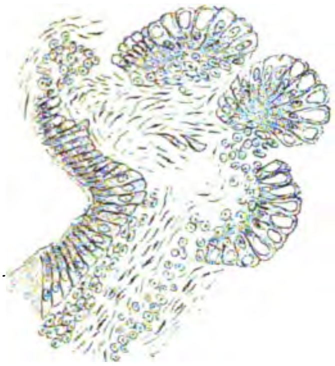
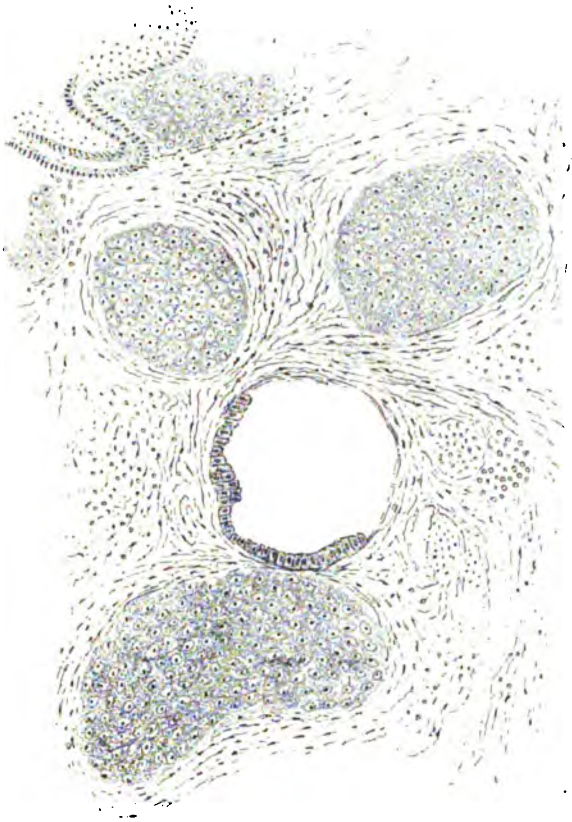


FIG. 3.



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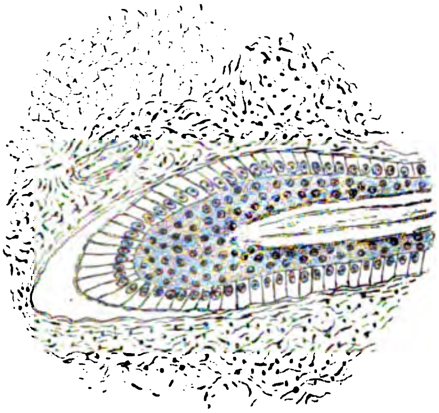

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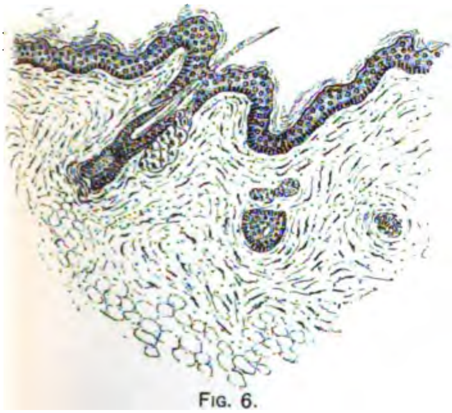

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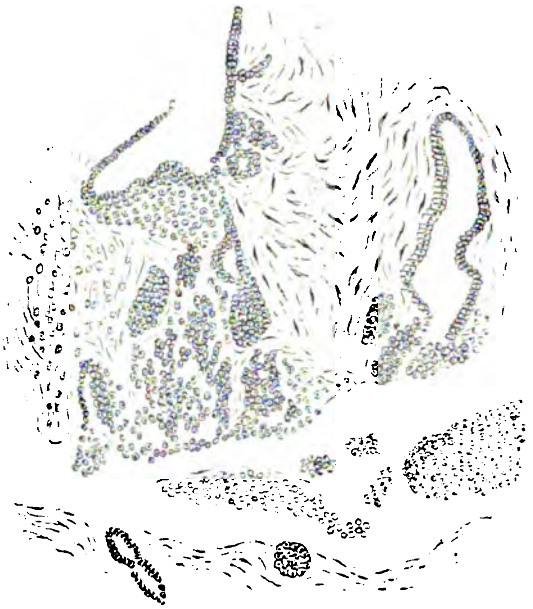

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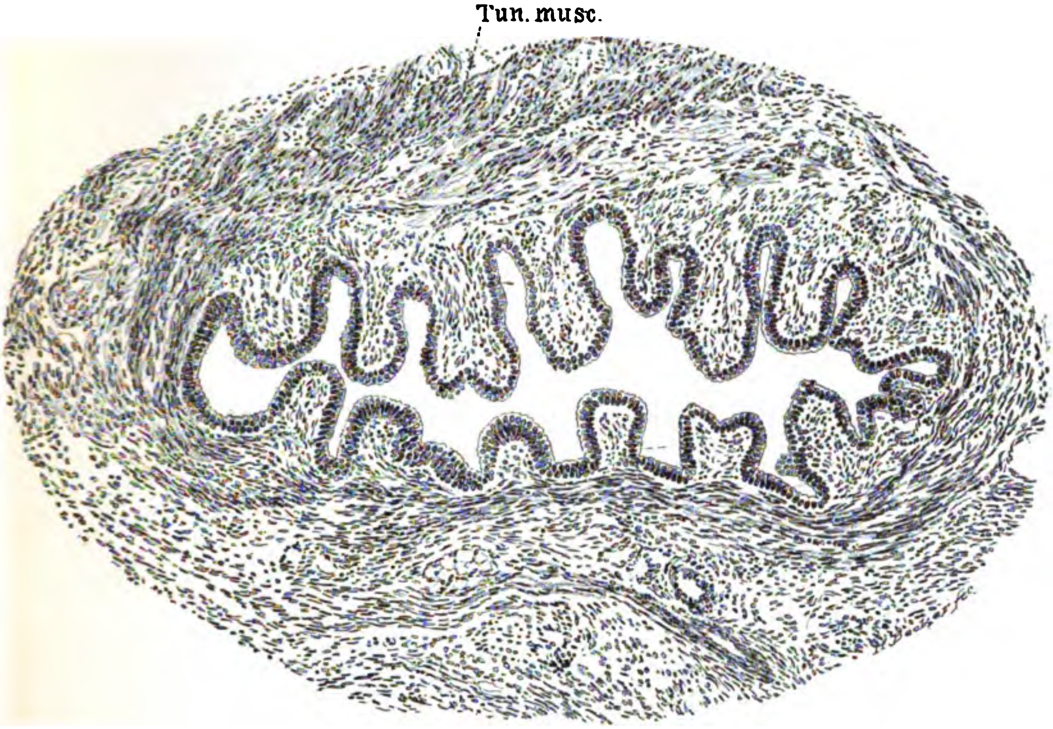

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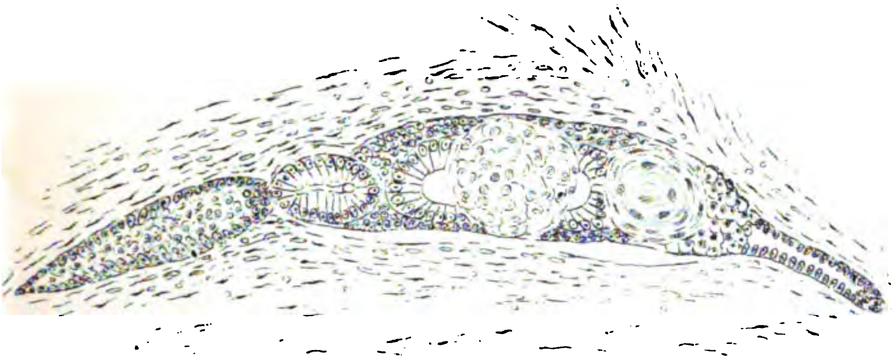

FIG. 9.

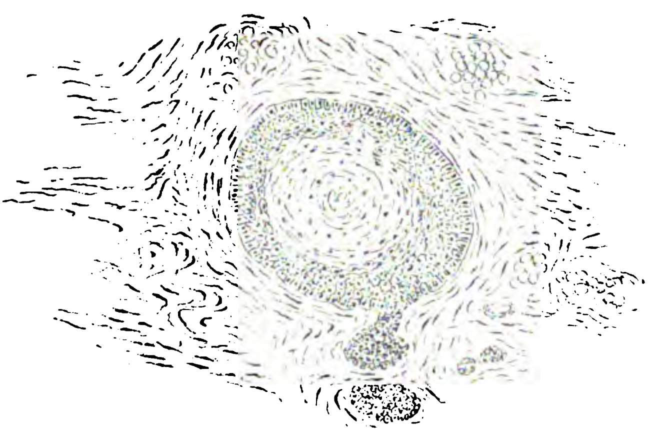

FIG. 10.

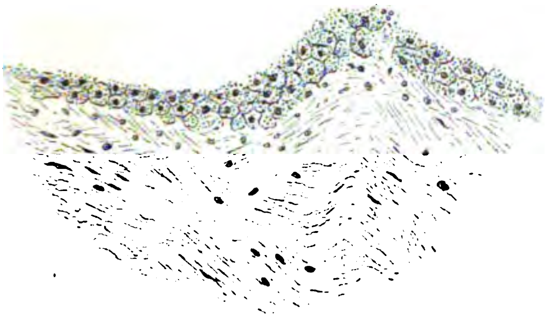

FIG. 11.

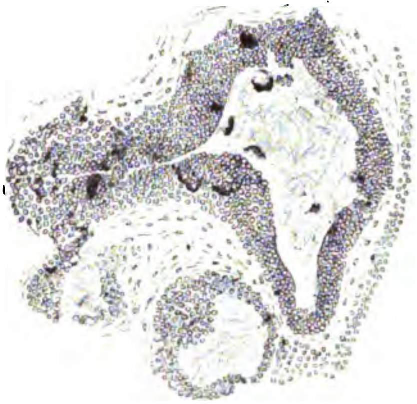

FIG. 12.

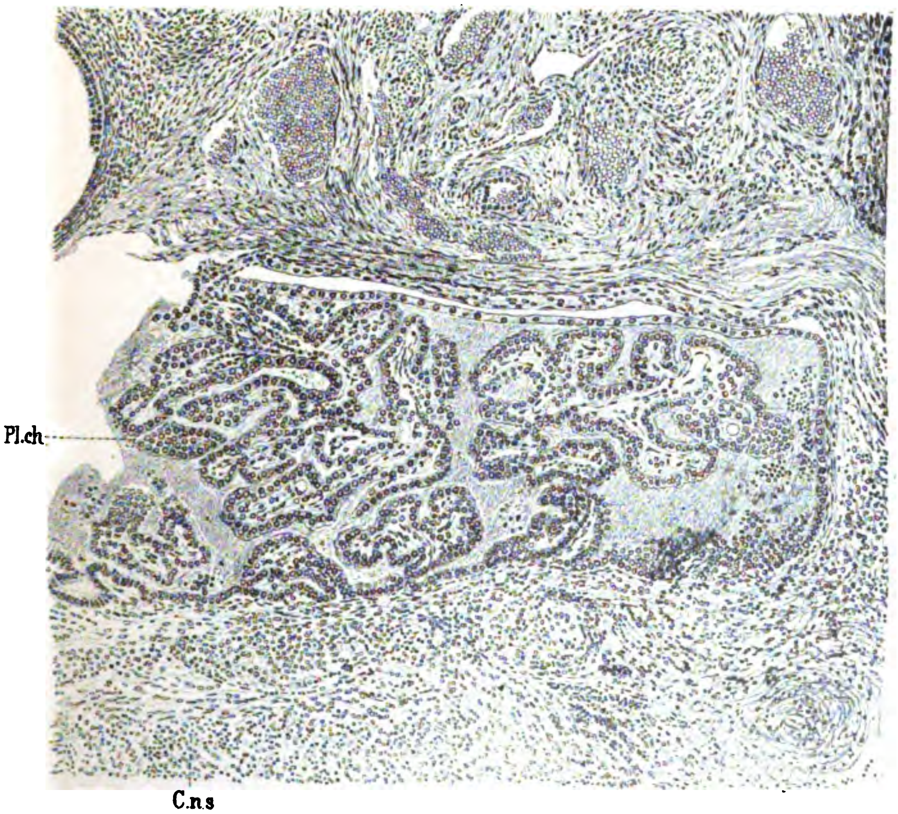

FIG. 13.

AN EXPERIMENTAL STUDY OF FAT STARVATION WITH ESPECIAL REFERENCE TO THE PRODUCTION OF SEROUS ATROPHY OF FAT.

By C. A. HERTER, M. D.

PLATES XXIII AND XXIV.

The experimental study which forms the basis of this paper was originally undertaken to determine whether the lesions of rickets or similar lesions can be produced in growing animals by withholding fats from their food as far as practicable. This inquiry was suggested by the alleged frequently low fat content of the milk of women whose children have grown rachitic on breast-milk, by the occurrence of rickets in children fed largely on condensed milk, which is poor in fat, and by the fact that the clinical indications of rickets are often lessened by a diet rich in fats.

The common pig, Sus scrofa, was the animal chosen for the experiment, partly because of his omnivorous habits and partly because the nutritive vigor of this animal seemed likely to render possible the extension of the necessarily depressing experiment over a long period of time.

CLINICAL SECTION.

Pig 1.—The first experiment was begun December 16, 1895, upon a boar about two months old weighing 24 pounds, and which will be referred to here as pig 1. This animal was fed exclusively upon milk furnished by the Walker-Gordon Milk Laboratory, from which the fat had been largely removed by the centrifuge. The composition of this milk as regards fat is remarkably constant, and analysis shows that it contains less than $\frac{1}{20}$ per cent of fat. The milk of the sow normally contains a high percentage of fat, the amount ranging from 8 to 10 per cent. The usual content of cow's milk skimmed by the ordinary method is about 1 per cent. The milk fed to pig 1, therefore, contained less than $\frac{1}{200}$th part of the fat which is found in sow's milk, and about $\frac{1}{20}$th part of the fat contained in ordinary skimmed milk. In other respects the milk did not differ greatly in composition from that of cow's milk, the content of milk-sugar being about 6 per cent, that of proteids about 4 per cent.

The duration of the experiment was 51 weeks. The daily quantity of milk allowed first was 1400 cc. This was increased from time to time until at the end of 46 weeks the total daily amount was 4000 cc. The feeding was usually in two portions, towards the end often in one. During the first six weeks the initial weight of the animal was just maintained; then during a period of about six weeks there was a moderate loss, the weight dropping from 24 to 19½ pounds. After this an increase in the food supply was followed by a steady gain in weight, so that 12 weeks after the beginning of the diet the animal weighed 2½ pounds more than at the beginning. 16 weeks from the beginning the weight showed an increase of 5 pounds, in 20 weeks an increase of 7½ pounds, and in 50 weeks an increase of 16 pounds, in all from 24 to 40 pounds. Within a week from the beginning the fæces were observed to be lighter in color and firmer in consistence than when the animal was on a mixed diet containing cereals. Very soon there was distinct constipation and the fæces were passed as small hard spheroidal masses. This constipation continued to the last. The fæces were regularly alkaline in reaction. The quantity of urine passed varied closely with the amount of milk given. The specific gravity varied between 1011 and 1014 in 24-hours' collections. The reaction was invariably distinctly acid—a notable peculiarity, as the urine of pigs on a general diet is almost always neutral when passed. During the first 8 or 10 weeks the animal seemed to be in every way normal except that he did not gain in weight. After 18 weeks it was observed that the animal's hair and skin were distinctly drier than is usual in pigs of this age, and that the hair was decidedly more scanty than that of a control pig of the same age. After 30 weeks these conditions were more pronounced, and some weakness of the hind legs was noted when the animal stood. From about this time it was noticeable that the animal lay on the floor of his cage a good deal of the time, and often dozed during the day, though he did not usually sleep. After 50 weeks he was quite unable to stand on his hind legs, which were thrown forward as the animal lay. There was no attempt whatever to use these extremities in walking. Some power remained in the hip muscles. The muscles were flabby but showed no indication of wasting. On the contrary the legs were puffy in appearance, although there was absolutely no pitting on pressure This condition had doubtless been present some time before it was noted The knee-jerks were present. The forelegs were weak, but the animal was able to walk by use of them. For a few days before his death the animal had a rise in temperature, his breathing was rapid and labored and he was cyanosed. When it was evident that death was impending he was killed. An examination of the blood just before death showed the hæmoglobin to be reduced to 65 per cent by Fleischl's instrument. The proportion of small red blood cells seemed increased. The proportion of white to red cells was normal. The blood count showed a slight reduction in the total number of red cells to the cubic millimetre.

Pig 8.—A second experiment was conducted with another boar weighing 26 pounds, and referred to here as pig 8. This animal was fed exclusively upon milk of the same composition as that used in the case of pig 1, but was given during the first twelve months of the experiment as much of the food as he was able to take, instead of being limited to a fixed quantity. The quantities consumed by him were considerably in excess of the amounts allowed pig 1—5, 6 or 7 litres per day. Toward the end of the first year the quantity of milk consumed was from 8 to 10 litres daily; from the end of the first year until the termination of the experiment the quantity was reduced to two litres per day. The duration of the experiment was 66 weeks. At the end of 12 weeks the skin was observed to be dry and scaly, and the hairs were scanty. At the end of 20 weeks some weakness of the forelegs was shown in standing, and the animal was inclined to sleep a good deal of the time. After 30 weeks there was marked weakness in all four legs. The appetite remained good. The urine and fæces showed the same peculiarities as were noted in the urine and fæces of pig 1. From time to time in the course of this experiment bits of skin and subcutaneous fat were removed from the back of the neck in order that their structure might be compared with a piece removed before the animal was put on this diet, and with the skin of pig 1. The weights of the animal were as follows: Nov. 12, 1895, 28 pounds; Jan. 21, 1896, 42 pounds; February 29, 55 pounds; April 21, 62 pounds; Nov. 23, 106 pounds. The gain in weight was thus much greater than in pig 1. On the reduction of the milk to 2 litres daily there ensued considerable loss in weight, and one week before death the animal was quite unable to stand owing to weakness. He was killed by bleeding.

Pig 9.—A third experiment was conducted on a pig weighing 24 pounds, with a view especially to a study of the chemical characters of the urine and fæces during the starvation. After 6 weeks the animal was again allowed an abundance of fat, and after two weeks this was again cut off. This animal is referred to as pig 9.

Pig 2.—At the time when pig 1 was put on a fatless diet, another animal, here known as pig 2, of about the same age and weight, was placed upon a wholly different diet, in which the quantity of proteid and of fat was much reduced, but in which an attempt was made to compensate for their reduction by the use of very large amounts of carbohydrate food, first in the form of Mellin's food, later as unrefined sugar. The results obtained from the study of this animal bear directly on the question of fat starvation, and in some ways the experiment may be regarded as a control to the others. The duration of this experiment was 4 weeks longer than in the case of pig 1, namely, 56 weeks There are several respects in which a comparison between the two animals is instructive. The urine, instead of being distinctly acid, as was regularly the case with the urine of pig 1, was neutral or alkaline at first, and later slightly acid in reaction. Toward the end of the experiment the urine at times contained sugar in small amounts soon after the administration of the richly saccharine food. The fæces, instead of being hard in consistence and light in color, as in pig 1, were usually dark in color and semi-fluid in consistence, and part of the time were diarrhœal in character.

More important clinical differences relate to the general condition of the animals. Although there was an even greater retardation of growth in pig 2 than in pig 1, in the former the skin remained soft and apparently well nourished, though a little dry. There was some falling out of the hair, but that which remained was abundant in comparison with the hair of pig 1. The animal appeared fairly well nourished, though small. There were no hæmorrhagic spots in the skin. A few days before the animal was killed he seemed rather feeble in all four legs, as shown by difficulty in standing, but he developed no distinct paralysis There was no swelling of the feet as in the case of pig 1.

To sum up: it was found that a growing pig which lived exclusively on nearly fatless milk for about one year was retarded in growth, suffered markedly in nutrition, and developed a hæmorrhagic eruption* and marked weakness of the extremities; that another pig kept on the same diet for 66 weeks developed the same clinical conditions excepting hæmorrhagic spots, but in less marked degree; and that a third animal which lived on a diet much restricted in proteids and fats but with a great excess of carbohydrates, though more retarded in growth, suffered distinctly less in general nutrition and apparently in a different manner in several respects.

* It seems not unlikely that certain of the lesions noted in this animal, especially the hæmorrhagic ones, were scorbutic in character.

PATHOLOGICAL ANATOMY.

Skin and Adipose Tissue of Pig 1.—The body is poorly nourished; there is a fairly abundant hæmorrhagic eruption on the lower part of the abdomen and into the skin of the perineum. Over the upper part of the sternum is an ulcer about 2 cm. square (clearly from pressure). On cutting through the skin it is seen that there is nowhere any subcutaneous fat; in place of this is a homogeneous, translucent layer of gelatinous-looking material, somewhat less thick than the layer of subcutaneous fat in a normal pig of the same size. On the back of the neck, near the median line, where the fat is normally very thick, this material forms a layer about 22 mm. in thickness. It may be stated here that in all parts of the body where fat is normally present a similar replacement of it by this material is evident, as about the heart, about the kidneys, between the muscles, in the omentum and elsewhere. At first sight the appearance of the material suggests that of œdematous connective tissue, but the substance appears quite homogeneous and yields no fluid on being cut into small pieces and compressed. This replacement of fat by the material described is the most striking and important pathological change noted in this animal. The microscopic characters of the skin are normal. The subcutaneous fat-cells are found to be so transformed as to be almost unrecognizable. The gelatinous layer consists chiefly of spheroidal and ovoidal cells (Plate XXIV), the average diameter of which is from one-third to one-sixth that of normal fat cells (Plate XXIII). The cells consist of a cell membrane, frequently irregular in outline, containing a homogeneous substance which stains rather faintly with hæmatoxylin and most basic aniline dyes. Each cell contains a nucleus which stains deeply with hæmatoxylin and resembles that of a young fat cell. These nuclei are not applied closely to the cell membrane, but may occupy any portion of the cell body. The intranuclear structure was not studied. Often the nucleus is surrounded by a small or considerable amount of finely granular material. Not infrequently what appears to be a vacuole of considerable size is found in the cell body. Sections from material subjected to the action of osmic acid, before further hardening and embedding, show no evidence of the presence of fat—they are wholly unaffected by this reagent. Running in from and continuous with the connective tissue of the skin are bundles of various sizes formed by connective tissue fibres. Frequently the smaller bundles separate into the individual wavy elastic fibres of which they consist, and these pass in all directions between the cells.* The cells do not lie close together as in normal fat tissue, but are separated from one another in places. Their appearance suggests that the intercellular spaces may have been occupied by some material which has been lost during the process of hardening or embedding. Between the cells with unbroken cell membranes sometimes lie processes which may consist of broken cell membrane. The capillaries are fairly numerous. Some of them have blind ends. Unfortunately the material from pig 1 was not examined fresh.

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* The elastic fibres are very abundant, but this is normally the case in the skin from the region of the ligamentum nuchæ.

Before describing other changes noted in pig 1 it will be convenient to refer to the changes found in the skin in pig 8 and in pig 2.

Subcutaneous Fat in Pig 8.—In this pig, skin from the back of the neck was removed from time to time. After nine weeks it is noticeable that there is some shrinkage of the fat cells, and that the cells in places are irregular in form. After fourteen weeks the fat cells are shrunken to two-thirds or one-half of their normal size; the outline of the cell membrane is often exceedingly irregular; the cell contents consist of larger and smaller droplets of fat which stain well with osmic acid, and it is noticeable that some of the nuclei are no longer in apposition with their cell walls. Twenty weeks after the beginning of the diet the fat layer appears pale. Although it does not look quite like normal fat, it does not appear gelatinous. The fat cells show more reduction in size than in the preparations referred to above. This is more pronounced in some lobules than in others. A few cells are seen which are not more than one-quarter or one-fifth the average diameter of normal cells. In preparations cut from pieces immersed in a 1 per cent solution of osmic acid the fat cells nearest the edge of the section have stained like normal fat cells, but there is a line of cells a little farther from the edge to which the osmic acid has penetrated less freely. Here the contents of the cells have stained only in spots, corresponding to the fat droplets already referred to. Between the droplets is seen in places a very finely granular or homogeneous material which does not stain with osmic acid, but tinges faintly with hæmatoxylin. This certainly is not fat, and it is possible that it is the accumulation of such material in larger amounts both in and between the cells which gives rise to the gelatinous appearance noted in pig 1. The nucleus in many of the cells lies well within the cell body. The connective tissue which lies between the groups of cells shows a distinct inclination to the separation of the individual fibres of which it consists—an unraveling, as it were, similar to but much less pronounced than that observed in pig 1.

The only additional change observed in the subcutaneous fat of this animal at the end of 60 weeks is a further considerable reduction in the size of the fat cells. In some places the subcutaneous fat has almost wholly disappeared; in most places it is from ⅛ to ¼ in. in thickness. Nowhere has there been any replacement of fat by distinctly serous material. A majority of the fat cells are simply much atrophied, but a few show the alterations described as present after the lapse of 20 weeks.

Subcutaneous Fat in Pig 2.—An examination in this case shows that the skin itself is normal in structure. The subcutaneous layer of fat is much reduced in thickness, being only about one-quarter the thickness of the fat layer from the corresponding portion of the back of the neck in a normal pig of the same age. The gelatinous appearance which was noted in the skin of pig 1 is wholly wanting, but the fat is perhaps a little paler in tint than that of normal animals. Minute examination shows that the fat cells are somewhat reduced in size, perhaps to two-thirds or three-quarters of their normal diameters. The contents of the cells are everywhere homogeneous; nowhere is there to be seen the breaking into fat globules noted in the sections from pig 8. The cell contents stain faintly with hæmatoxylin. With osmic acid they stain exactly like normal fat cells. The connective tissue separating groups of fat cells is quite normal in appearance. Between the individual fat cells are more nuclei than are normally present in an animal 15 months of age, but not more than are seen in the subcutaneous fat of pigs a few weeks old.

The appearances, then, are chiefly those which we might expect to find as the consequence of the arrest of fat formation in a young animal. The reduction in the size of the cells is doubtless to be regarded as the result of a process of simple atrophy. Notwithstanding the long period over which the experiment extended, there is nothing to suggest even the beginning of the transformation which was noted in pigs 1 and 8.

Examination of Pig 1 continued.—On opening pig 1 much straw-colored fluid is found in both pleural cavities, in the pericardium, and in the peritoneal cavity. The parietal pleura on both sides is the seat of a moderate number of small superficial hæmorrhages; the lower lobe of the right lung is partially consolidated, and on section presents numerous larger and smaller infarctions. On the pleural surface over the consolidated area is a thin layer of fibrin. The base of the left lower lobe is congested, but there is no consolidation. Microscopically the consolidated lung presents numerous infarctions with surrounding pneumonic consolidation. The heart is large, pale in color, and flabby in consistence. The weight of the heart is 232 grammes. That of a normal animal of the same age, and weighing five and one-half times as much, is only 358 grammes, or about one-half as much more. On the anterior surface on either side of the coronary artery are several hæmorrhages of moderate size, extending about one-quarter of an inch into the myocardium. In place of the subpericardial fat usually found is a layer of the gelatinous tissue above described. On section the myocardium is pale and the wall of the left ventricle is notably thickened. The valves are normal. There are several small endocardial hæmorrhages in the left ventricle. The coronary arteries were not examined. Microscopic examination shows that many of the muscle fibres of the left ventricle are the seat of slight granular degeneration. In places there are small areas of richly cellular connective tissue. The liver is normal in appearance and consistence. The gall-bladder is distended with light-colored bile. The bile duct is pervious. The histological appearances of the liver are normal. The mucous membrane of the stomach is normal in appearance. In the cardiac portion there are a few hæmorrhagic spots in the mucosa, and one small superficial ulcer. The mucous membrane of the large and small intestine is normal in appearance. The pancreas is normal in consistence, yellowish in color and disproportionately large in comparison with the body weight. Histologically it appears to be normal. The spleen is small, rather pale, and normal in structure. The kidneys are surrounded by a considerable amount of hyaline gelatinous material of the same character as that beneath the skin and representing altered adipose tissue. The kidneys are disproportionately large in comparison with the body weight. The capsules strip off readily. The surfaces are smooth, slightly paler than normal; the striations are almost obliterated and the cut surface is studded with numerous minute hæmorrhages. The chief histological changes observed in the kidney are as follows: The epithelium of the tubules is everywhere the seat of granular degeneration, the changes being slight but distinct in the collecting tubes and in Henle's loops, and considerable in degree in the secreting tubes. The cells of the secreting tubes are somewhat swollen and in some cases desquamated. Many degenerated cells fill the tubes. In other cases the contents of the tubes consist of the nuclei of degenerated cells and a granular detritus. Frequently the secreting tubes contain hyaline material which stains with eosin. Nowhere do the tubes seem much dilated. The tufts are somewhat swollen and usually occupy the entire capsular space. They appear more richly cellular than normal. The epithelium covering the tufts is in places swollen and granular, that lining the capsule appears normal. The capillaries of the tuft generally appear compressed. The vessels everywhere seem normal. No alterations can be detected in the connective tissue. In short, the appearances are those of a chronic parenchymatous degeneration. The adrenals are normal in consistence but are distinctly increased in size, their weight being as great as in a normal pig whose body weighs more than five times as much as that of pig 1. On section the cortex appears thickened and the medulla is abnormally dark, suggesting the presence of hæmorrhage, but this view is not confirmed by microscopic examination. The radially disposed columns of cells in the cortex appear swollen, and in places the cell bodies are less granular than normal. The nuclei seem unchanged. In the medullary portion the ganglion cells have become greatly swollen and in some cases are almost wholly replaced by large vacuoles. Through an oversight the thyroid was not removed.

The knee-joints are found to be filled with bloody and viscid synovial fluid. The bones of the skull are porous, very brittle and distinctly thinner than those of a normal animal of the same size. The femurs are about normal in weight. The bone marrow in each femur is replaced wholly by material which has the appearance and consistence of a blood clot. Unfortunately this material was not successfully hardened, and no satisfactory examination was made of it. The remaining long bones were not examined.

The central nervous system was not examined histologically. Of the skeletal muscles pieces only of the quadriceps femoris were examined histologically. Sections of the muscle show that a majority of the muscle fibres have undergone a marked reduction in size. While there is an apparent increase in the sarcolemma nuclei, there is probably no actual increase in their number. A few fibres are reduced to a width not greater than the diameter of their nuclei. The striations of the fibres are as a rule well preserved. The fibres stain well with eosin, and on transverse section some of them have a vitreous appearance and look as though broken into large fragments. There is no increase in connective tissue. The intermuscular fat shows the same peculiarities that have been noted in the case of the subcutaneous fat. All the visible vessels show an increase in the thickness of their walls, which is in some cases very marked and implicates chiefly the muscular coat.

The post-mortem notes on pig 2 are as follows: There is little or no subcutaneous fat; the lungs are normal; the heart is normal in size, color and consistence; the muscles are a little soft; the liver is normal in appearance and consistence; the pancreas is rather hard; the kidneys are normal; the striations of the cortex are very plain. The femur, cut longitudinally, shows the development of bone to be almost exactly the same in degree as in the case of pig 1. In thickness and consistence the bone is not perceptibly different from that of pig 1. The marrow adjacent to the compact bone is red in color; it has the appearance presented by *all* the marrow of pig 1, whilst the remaining and greater part of the marrow, that occupying the central portion, is of the normal light pink color. The hæmoglobin was estimated immediately after death. The reading with Fleischl's instrument was from 100-105 per cent.

Examination of pig 8.—The animal is considerably emaciated. The subcutaneous fat is everywhere either entirely absent or greatly reduced in amount. The muscles are normal in color and consistence. The pericardium contains almost two ounces of clear straw-colored fluid. The heart is flabby in consistence and pale in color. Hæmorrhages into the heart muscle are not noted. The liver, spleen, pancreas and kidneys appear normal. The epi- and pericardial fat presents the same gelatinous appearance observed in pig 1. The same change is very pronounced in the adipose tissue about the kidneys and the ureters and in the omentum. Microscopic examination shows that the atrophic adipose tissue about the heart, kidneys and elsewhere presents essentially the same appearances as those already described as occasionally present in the adipose structures of pig 1. The only difference worthy of mention is that there is visible an abundant homogeneous intercellular matrix, which probably consists of an albuminous serous material. Aside from the alterations in the adipose tissue the following changes are noticeable. The skeletal muscles show granular degeneration of the muscle fibres in places; other fibres exhibit different grades of simple atrophy. In the cortex of the pancreas are numerous hæmorrhages of moderate size, and in places free brown granular pigment. In some areas the epithelium is the seat of granular degeneration. The secreting tubes of the kidney show marked granular degeneration of the epithelium; they contain some granular debris and fibrin, and some hyaline and granular casts. The epithelium of the loops and collecting tubes shows granular degeneration; the tubes contain much hyaline material and some granular casts. The capsular epithelium is swollen and slightly degenerated. Sections from the motor cortex stained with eosin and hæmatoxylin and by Nissl's method show degenerative changes in a considerable proportion of the nerve cells. The outer layers of the cortex are œdematous. The cortex of the frontal region shows similar changes. In several places there are small hæmorrhages into and beneath the pia of the central and frontal convolutions.

PATHOLOGICAL CHEMISTRY.

In the case of pig 1 an endeavor was made to determine the nature of the subcutaneous gelatinous material which has been described. With a view to determining the presence of mucin the tissue was extracted with lime water, mucin being soluble in dilute alkalies. It was found that on the addition of dilute acetic acid to this extract there occurred a considerable flocculent precipitate. Up to this point the behavior of the material is that of a mucin-containing substance. When, however, a 2 per cent solution of acetic acid was added to the precipitate the latter was again dissolved. In this respect the extracted material no longer corresponds to the reactions of mucin. Its solubility in a 2 per cent solution of acetic acid is a characteristic which removes it from the group of mucins, which are not soluble in an excess of acetic acid. Moreover, on decomposition with dilute sulphuric acid it failed to yield a reducing substance such as is obtained in the case of mucin. The mucins are glucosides, that is, compounds of a true proteid (generally a globulin) with animal gum, which by treatment with dilute sulphuric acid are converted by hydration into a reducing substance. On the other hand the lime water extract gave the characteristic xanthoproteic reaction and the reaction with Millon's reagent. This is conclusive evidence that the body under examination is not, strictly speaking, a mucin, but is a true proteid, and probably places it in the group of phosphorus-containing proteids known as nucleo-albumins. Unfortunately the phosphorus content of the material was not determined. Positive proof that it is a nucleo-albumin is therefore wanting.

Before discussing the subject of fat atrophy we may pass to the consideration of some nutritive disturbances which have been noted in the animals that were subjected to fat starvation. As already stated, there was some reason to think that fat starvation might lead to the production of rickets or a condition resembling rickets. For this reason attention was paid to the study of the salts of the urine and fæces, and the results noted were of sufficient interest to induce a continuation of this class of observations even after it became clear that the lesions produced were not those of rickets.

Looking first at the urine, we find that in the case of pig 1 on Dec. 24, 1893, one week after the beginning of the fatless diet, the urea excreted during the 24 hours was 8.047 gm., whilst the P_2O_5 excreted in the same period was 1.175 gm., the ratio of P_2O_5 to urea being 1 to

6.8. The ratio in health, on a milk diet, varies from about 1 to 6 to 1 to 14. On Jan. 4, 1894, the urea was 18.142 gm., the P_2O_5 1.190 gm., with a ratio of 1 to 15.2. On January 30 the urea was 30.895 gm., the P_2O_5 1.023 gm., ratio 1 to 32.4. On April 20 the urea was 25.039 gm., the P_2O_5 1.065 gm., ratio 1 to 23.5. Finally on Nov. 24, 1894, a few days before the completion of the experiment, the urea was 38.626 gm., the P_2O_5 0.759, ratio 1 to 50.9. Looking these figures over it will be seen that there was a very considerable increase in the amount of urea excreted between the beginning and end of the experiment, an even greater increase than was to be expected in a young and growing animal. But while this occurred in the urea excreted there was not a corresponding increase in the excretion of P_2O_5. Far from there being any increase in the absolute amount of P_2O_5 excreted, the figures indicate an inclination to an absolute decrease. Under ordinary conditions both in pigs and in man the excretion of P_2O_5 shows a rough correspondence with the urea excretion in any fluctuations that may occur. In the diet of pig 1 the P_2O_5 of the milk was increased proportionately with the proteids of the milk. Hence we should expect to find a more than ordinarily close correspondence between the excreted urea and the P_2O_5. On the contrary we have ratios fluctuating from 1 to 6.8 to 1 to 50.9.

The observations on pig 8 were unfortunately not begun until the fatless diet had been continued for nearly 8 weeks. There is lacking, therefore, a study of the early period in which it is likely that the most pronounced deviation from the normal ratio of P_2O_5 to urea might be expected to occur. The results are, however, not without interest. Between January 9 and April 8, the period of observation, the urea fluctuated irregularly between 20.38 gm. and 42.64 gm. daily, whilst the P_2O_5 varied from .71 gm. to 1.93 gm. The ratios fluctuated irregularly between 1 to 16.70 and 1 to 48.77, with no material difference between the ratios at the beginning and at the end of the period, but with perhaps an inclination to lower ratios at the end than at the beginning. The striking feature of the observations is the high ratios throughout, or in other words the small amount of P_2O_5 in proportion to the urea.

An examination of the ratios in the case of pig 9 leads one to suspect that in pig 8 there may have been a larger excretion of P_2O_5 at the beginning of the fatless period than later. In this case the first observation on the urine was made on February 26, 1896, when the animal had been for four days on centrifugalized milk. The urea increased irregularly, but on the whole gradually, from 4.86 gm. on Feb. 26 to 29.47 gm. on April 11 and 20.32 gm. on April 13. During this time there was an irregular but relatively slight increase in the P_2O_5, so that the ratios increased from 1 to 10.94 at the beginning to 1 to 32.40 at the end of the period.

We thus have records of three animals fed on nearly fatless milk, in all of which the excretion of P_2O_5 is very distinctly lower than in animals fed on milk containing a natural proportion of fat; in two of these cases the study of the urine was begun sufficiently early in the experiment to permit of the detection of a marked fall in the excretion of P_2O_5 as a result of the continuation of the fatless diet.* In the milk on which these animals were fed, which has been shown to be remarkably constant in composition, it is presumable that the P_2O_5, present chiefly in salts of Ca and Mg, bore a fairly constant relation to the N present in the proteids of the milk. The N of the urine and the P_2O_5 of the urine are admitted to be ultimately derived from the food ingested. How then are we to explain the fact here noted that the P_2O_5 excretion did not keep pace with the N excretion? The P_2O_5 unaccounted for must be assumed either to have been retained in the body, probably as the Ca and Mg salts of growing bone, or to have been lost through the fæces owing to defective absorption. As the latter explanation was thought to be more probable it was determined to study the effect of adding a considerable amount of fat in the form of suet to the usual quantity of fatless milk consumed by pig. 9. One hundred grammes of suet, therefore, were given daily with the milk. The feeding remained the same in all other respects. The effect on the urine was immediate and striking. The absolute amount of the P_2O_5 increased rapidly to a somewhat higher average than any previously observed in the course of this experiment, in spite of the fact that the absolute urea excretion fell distinctly. The result was an immediate return to the ratios normally observed in pigs on a mixed diet. This may be looked upon as strong evidence in favor of the view that the low P_2O_5 excretion observed in these experiments is the result of defective absorption of phosphates from the intestine. This assumption is further reinforced by a study of the excretion of the salts through the fæces. In pig 1 we find that the ash of the fæces took on an increase from 16.96% of the solid matter of the fæces on Dec. 24, 1893, to 47.48% on Jan. 13, 1894, to 49.92% on April 10 and 41.33% on Oct. 5. The first figure is within the normal limits on a mixed diet; the remaining percentages are all distinctly high. In pig 8 the figures representing the percentages of the salts are all very high, ranging as they do from 42.53% to 70.89%. In this case the percentages are only a little higher at the end of the period of observation than at the beginning, but it should be remembered that the analyses were not begun until the animal had been nearly 8 weeks on a fatless diet. In pig 9 again it is found that the percentages of the salts run high throughout (from 36.16% to 54.95%), but in this case the observations were begun two weeks after the beginning of the experiment, and there is a very evident gradual increase in the percentages of the salts lost by way of the bowel. It is interesting to note in this connection that on the addition of fat to the diet of pig 9 there was a prompt fall in the percentage of salts passed with the fæces. Of course the mere fluctuations in the fæcal fat would account for a considerable fluctuation in the percentage of ash, but not for the marked differences here noted. It is further noticeable that the percentage of P_2O_5 and of Ca of the total salts shows a slight progressive increase in both pig 8 and pig 9, which of course corresponds to a considerably greater increase in the absolute P_2O_5 and CaO which is lost through the fæces. The results obtained make it clear that the presence of fat in the diet exerted an important influence on the absorption of salts, especially those of P_2O_5 from the intestine, in the animals experimented upon. They show, at least in the case of pigs, that fat starvation entails a marked diminution in the absorption and in the renal excretion of P_2O_5. Whether the same relationship holds good generally in the higher mammals cannot be stated, but it seems likely that it is very generally operative as a physiological principle. I have several times noted the presence of an unusually low P_2O_5

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*The fall in the absolute amount of P_2O_5 in pig 1 may perhaps be explained by the fact that the quantity of milk received by him was relatively small. Pigs 8 and 9 show no absolute fall in P_2O_5. Pig 8 was given an unlimited quantity of milk, pig 9 was on a fixed amount, but this was considerably larger than in the case of pig 1.

content of the human urine when the quantity of fat ingested was much restricted. This suggests that the same relationship applies in the human subject. At present I know of no satisfactory explanation of this interdependence.

Whether the deficient supply of salts to the organism is a factor in producing the fat atrophy here described must be regarded as exceedingly doubtful. It is much more likely that its chief effect is to produce the striking retardation in the development of the skeleton which was noted in pigs 1 and 8. Of interest in this connection is the fact that the analysis of the solids of the femur gave essentially the same percentages of P_2O_5, CaO, and of ash as the femur of a normal pig of the same age but of much greater skeletal development.

There are other features of interest in the comparison of the analyses made of the various organs of pig 1 with those made upon the organs of a normal animal, but I shall here mention only a few of the more striking results. Thus it is notable that the percentage of fat in the femur of pig 1 was only .48% of the solids, whilst that in the femur of a normal pig was 2.85% or almost six times as much. The percentage of fat in the femur of pig 2, the control animal, on a preponderating carbohydrate diet, was 8% of the solids or sixteen times as much as in pig 1. These figures well illustrate the general impoverishment of the body in fat. In the case of the liver the reduction is much less pronounced. The ether extract from the liver of pig 1 was 8.9% of the solids, from pig 2 it was 12.2%, and from a normal animal 12%. The preservation of so considerable an amount of fat in the liver is in accord with the experiments of Noël Paton, which showed that in pigeons subjected to a fast of four days there was no reduction in the normal amount of fatty acids in the liver, although any excess of fat stored in the liver was rapidly reduced to normal.*

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* Probably the object of this storing of fatty acids (as suggested by Paton) is to provide the necessary material for the synthesis of lecithin by the combination of phosphorus with fatty acids. The nucleins of the cells are constantly breaking down, and the store of fatty acids seems necessary to hold the P_2O_5 which results from this katabolism. See D. Noël Paton, On the Relationship of the Liver to Fats, *The Journal of Physiology*, xix (1896), 167.

It is also noteworthy that notwithstanding the very great reduction of fat in the body, the lecithins of the brain, which are phosphorized fats, were preserved in their normal proportions. This is shown by the following figures:

	Pig 1.	Pig 3 (normal).
Lecithins of grey matter,	2.6%	2.9%
Lecithins of white matter,	5.2%	4.3%

The chief features of interest in the chemical study of the blood were the fact that in pig 1 the serum globulin was markedly reduced in proportion to the serum albumin, and that the urea was considerably below the normal percentage.

The most obvious, and perhaps the most interesting, consequence of the foregoing experiments in fat starvation is the universal serous or gelatinous change in the subcutaneous and other fat depositories. From what has already been said of the microscopical and chemical characters of this altered adipose tissue in pigs 1 and 8, it seems reasonable to interpret as follows the process which leads to extreme changes like those noted in pig 1. After a few months of fat starvation the fat cells shrink somewhat, but their contents continue to consist wholly of fat. Next the contents of some of the cells break up into larger and smaller fat globules and a material between these globules which is not fat and stains feebly with hæmatoxylin and basic aniline dyes. This change becomes universal, the cells diminish very much in size, the cell membrane grows irregular in outline owing to the diminution in the volume of the cell body, and the nucleus is no longer applied to the cell membrane but may occupy any position in the cell. In time the much shrunken contents contain no fat whatever and consist merely of a small amount of homogeneous material which stains faintly with hæmatoxylin, and in some cases of a small amount of finely granular matter near the nucleus. Inasmuch as the transformed layer of adipose tissue maintains almost the volume of an abundant normal subcutaneous fat layer, in spite of the shrinkage of the cells to one-third or one-sixth of their normal size, and inasmuch as there is nowhere any evidence of cell multiplication, it is reasonable to suppose that the volume of the transformed layer is maintained in part by the presence of a newly formed intercellular substance. Of this the separation of the shrunken cells and the separation of the bundles of elastic fibres into their individual fibres may perhaps be regarded as evidence.

The appearances noted in the epicardial and perirenal fat of pig 8, where, probably owing to more successful preservation, an abundant intercellular substance was present, confirm the opinion here expressed in reference to the nature of the conditions in pig 1.

The histological changes which occur in atrophic adipose tissue have been made the subject of careful study by Flemming,* Czajewicz,† Schmidt‡ and others. Flemming in his classical studies of fat atrophy showed that when fat tissue undergoes atrophy, as seen in experimental starvation or in wasting diseases, the atrophic fat cells do not all undergo the same changes. In many cases three distinct types of atrophy could be distinguished. The commonest form of atrophy is that characterized by a general and evenly distributed reduction in the size of the fat cells, the cells undergoing no alteration either in contour or in the character of their contents. This condition is known as simple fat atrophy. It is often overlooked in the human subject. It is a usual accompaniment of a variety of nutritive and other disorders characterized by wasting.

Another type of fat atrophy is distinguished by cell proliferation. Here the small laterally placed nucleus of the fat cell, together with the small quantity of protoplasm by which it is surrounded, undergoes cell division. As the proliferative process advances the fat content of the cell becomes transformed and disappears. The newly proliferated cells were at one time thought to resemble leucocytes, and Waldeyer maintained that they actually were wandering cells. There are, however, several facts which are inconsistent with this view—the implication of isolated fat cells, the large size attained by the proliferated cells, their branching form and the manner in which the cell bodies sometimes remain connected with one another by slender trabeculæ after nuclear division has occurred. This process, which is especially apt to occur in fat cells where rapid and extreme wasting has occurred, is described by Flemming as atrophic proliferation (atrophische Wucherung).

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* Flemming's studies are by far the most important contributions relating to the subject of fat atrophy. The following are his chief writings upon this subject: W. Flemming, *Centralbl. f. d. med. Wissenschaft*, 1870, No. 31; *Arch. f. mikr. Anat.*, vii (1870), 32 and 328; Virchow's *Archiv*, lii (1871), 568 and lvi (1872), 146; *Arch. f. mikr. Anat.*, xii (1876), 391.

† F. Czajewicz, *Arch. f. Anat., Physiol. u. wissensch. Med.*, 1866, 289.

‡ H. Schmidt, Virchow's *Archiv*, cxxviii (1892), 58.

The process of atrophic proliferation differs strikingly from the third form of atrophy described by Flemming, namely, serous atrophy (seröse Atrophie) or gelatinous atrophy (gallertige Atrophie) in which the content of the fat cells is a homogeneous or finely granular material which contains no fat, and in which the transformed and atrophied fat cells are separated by a serous or gelatinous substance which is thought to be of an albuminous nature. This condition of serous atrophy has been observed by Flemming, Schmidt and others in atrophic fat in association with the condition of atrophic proliferation, but it often occurs independently. It has been found in the human subject in states of prolonged inanition, such as phthisis and cancer. The alteration is usually confined to the epicardial, perirenal and omental fat, but is sometimes found elsewhere.

The structural alterations which were observed in pigs 1 and 8 show no definite indications of the process of atrophic proliferation as it occurs in the atrophy due to the action of iodine, in that which follows the mechanical constriction of nutrient vessels or in the atrophy which occurs in rheumatic nodules.* Very few of the atrophic fat cells contain more than one nucleus, and there is nowhere seen the cell division which characterizes proliferative atrophy. Although the intercellular connective tissue is more cellular than that seen in normal adult fat, this seems to be due partly to arrest of development, partly to approximation of atrophied cells.

On the other hand, the appearances noted in pigs 1 and 8 bear so close a resemblance to the condition of so-called serous atrophy as it occurs in the human subject that there can be no doubt as to the identity of the processes. The propriety of the name "serous atrophy," implying as it does a replacement of the normal contents of the cell by serum, may well be questioned in view of the observations recorded in this paper. I have, however, continued to use this term as the one generally adopted to designate the same condition in human beings. Comparing the appearances seen in the gelatinous fat atrophy observed in the epicardial fat in wasting diseases, with those which I have produced experimentally, it is found that in both conditions there is a marked shrinkage of all the fat cells, with transformation of their contents, into the same serous or gelatinous material that constitutes the abundant intercellular substance. In both conditions the absence of fat is demonstrable by means of osmic acid. In neither state is the vascularity of the atrophic fat so great as in normal fat. Certain points of difference may, however, be recognized. In the atrophic fat cells of experimental origin only one nucleus is seen in each cell. Very rarely two nuclei, surrounded by a small amount of protoplasm, were demonstrated in the same cell; a larger number was not observed. In preparations made from atrophic pericardial fat, on the contrary, a considerable number of cells appear in which nuclear multiplication has occurred in the atrophic fat cell.

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* I have seen a beautiful example of fully developed atrophic proliferation in a preparation from a small mass of human omental fat which, as the result of an operation, had suffered constriction from the remaining omental fat during several days. I am indebted to Dr. Shelby, of the Loomis Laboratory, for an opportunity to study this material.

It may be stated, without fuller description of the histology of the proliferative process, that in such cases of serous fat atrophy as have come to my notice in the human subject, the process of atrophic proliferation has co-existed with the process of serous atrophy. In these cases the evidences of cell proliferation have existed in only a small proportion of the atrophic fat cells; the serous atrophy has been the preponderating, but not the exclusive process. In the atrophic fat which develops as the consequence of prolonged fat starvation we may regard the serous form of fat atrophy as the sole lesion. It seems probable, from a consideration of the literature relating to the atrophy of fat, that the experimental serous fat atrophy observed in the animals which form the subject of this paper is the type of serous fat atrophy that has been described.

It seems probable, on experimental grounds, that in pigs at least, serous fat atrophy can be produced only by prolonged deprivation of fat; the removal of carbohydrates, of proteids or of salts giving rise merely to simple atrophy, or to a limited and disseminated serous atrophy. It seems not unlikely that the advanced process of serous fat atrophy observed in the human subject, in the course of chronic tuberculosis and other wasting diseases, depends, similarly, on the presence of a very low percentage of fat in the blood.

Another question which suggests itself is whether the full explanation of the serous atrophy of fat starvation lies in the deprivation of fat to which the organism is subjected. Moreover, does fat starvation under all circumstances entail serous atrophy of fat tissues if the privation be continued long enough? In the case of pig 1 it will be remembered that the supply of proteid and carbohydrate food, though considerable, was restricted. May it not be that simple starvation is in part responsible for the observed changes? The fact that the same kind of changes, though less extreme, was going on in the skin of pig 8 while he was being given an unlimited supply of fatless milk of the same composition as that given pig 1, indicates that general starvation does not play an essential role in producing the beginnings of the condition.

The findings in the case of pig 2 make it clear that the removal of fat from the milk to the extent practised in these experiments does not necessarily induce the serous atrophy. In this animal, as already explained, there was even less fat and proteid ingested than in pigs 1 and 8, but an enormous quantity of carbohydrate food was given. As is well known, mammals have the power of converting carbohydrates into fat, probably chiefly in the liver and through the intermediate stage of glycogen formation, and it is in the highest degree probable that this is the explanation of the absence of the serous atrophy in pig 2. It is to be noted here that though the carbohydrate excess prevented the occurrence of serous atrophy, it was not capable of obviating the gradual absorption and simple atrophy of the subcutaneous fat.

There remains to be noted a collateral feature of considerable interest, namely, the chronic degeneration of the kidney which was observed in pig 1. The explanation of this which appears most probable is that the degeneration resulted from the long-continued excessive activity of the renal epithelium in the excretion of the N-containing constituents of the urine, which, owing to the highly nitrogenous diet, were excreted in pronounced excess. That such exaggerated physiological activity on the part of the renal epithelium may give rise to degeneration was conclusively shown, it is believed for the first time, by the following experiment:

> The left or larger kidney was removed from a healthy dog whose daily urea excretion ranged from 10 to 15 grammes on a sufficient diet. When the animal recovered from the operation he was placed on an excessive meat diet, with the result that the excretion of urea was increased to 30 or 40 grammes daily. The remaining kidney was thus compelled to do several times the work which it would ordinarily be required to do. The experiment was continued for one year, during which time the animal was kept in confinement but under otherwise hygienic conditions. He seemed well most of the time, but had occasional vomiting seizures and periods of loss of appetite. He had no symptoms of any acute infectious disease. Autopsy showed that the remaining kidney was perhaps slightly larger than normal; the cortex was rather pale and the markings indistinct, the epithelium of the secreting tubes was the seat of distinct granular degeneration, and there were occasional thin cortical wedges of young connective tissue. For several weeks before the animal was killed the urine had contained a trace of albumin but no casts. The kidney first removed was normal.

Another indication that the changes found in the kidneys of pig 1 were the result of their prolonged overwork is the fact that the kidneys of pig 2, whose nitrogenous food was much more limited than that of pig 1, showed no degenerative change. During the last days of pig 1 there were fever and some pneumonic consolidation, and though it is not likely that the renal changes were the result of infection, its occurrence necessitates caution in the interpretation of the degeneration.

CONCLUSIONS.

The following inferences may be made from the foregoing study:

1. The lesions resulting from fat starvation, at least in the case of pigs, do not resemble or even suggest those of rickets.

2. Prolonged fat starvation leads to the entire disappearance of fat from the adipose tissues. The form of fat atrophy observed as the result of experimental fat starvation corresponds to the serous fat atrophy described by Flemming, and is essentially the same type of fat atrophy as that found in the epicardial and perirenal fat in the human subject as the result of wasting disease.

3. The lecithins of the brain and the fat of the liver are not materially reduced by fat starvation.

4. Fat starvation does not lead to advanced serous fat atrophy of the subcutaneous fat if the animal be given a large excess of carbohydrate food or a considerable excess of the carbohydrate and proteid constituents of milk.

5. Fat starvation causes a very imperfect absorption of the salts of P_2O_5 from the intestine.

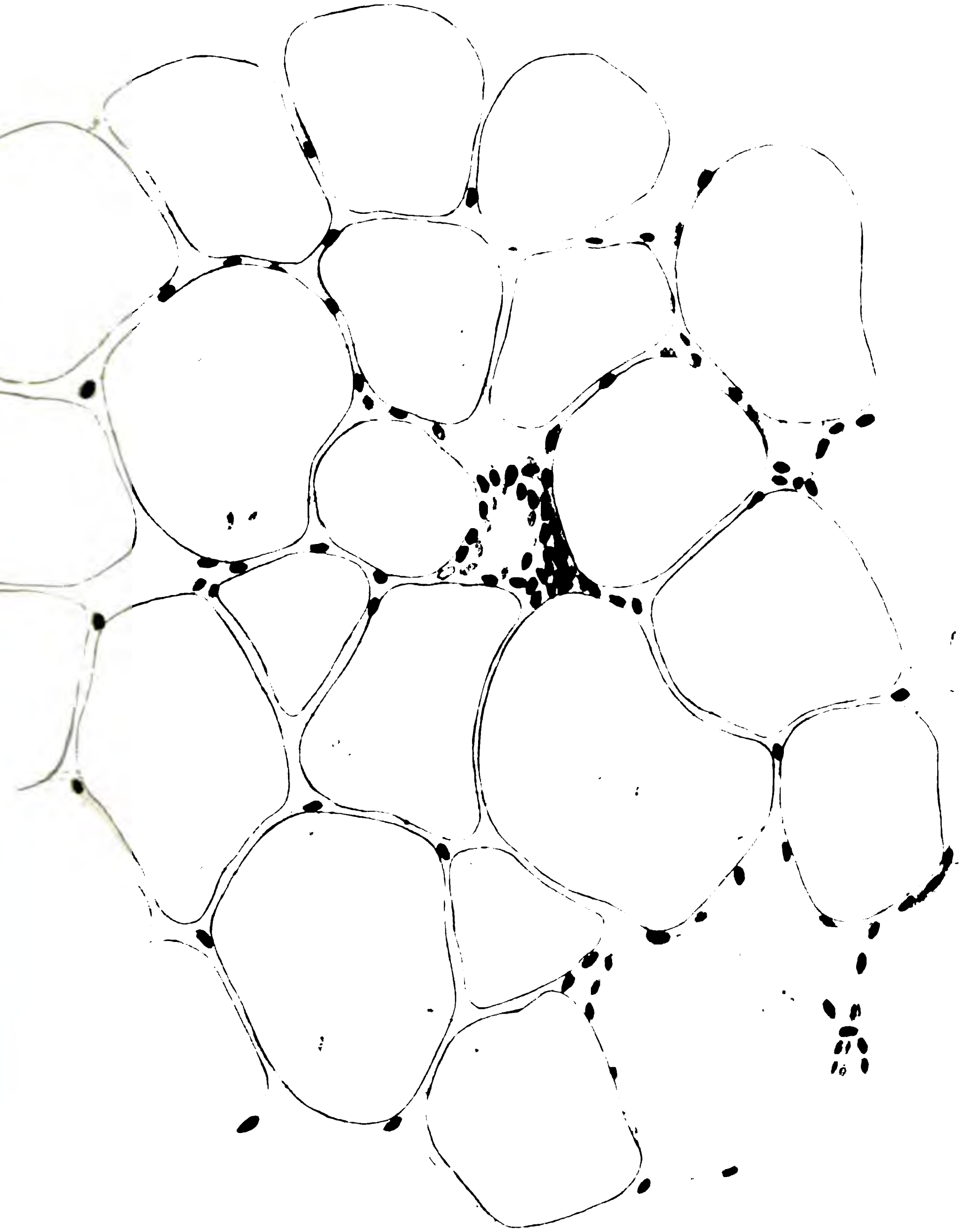
DESCRIPTION OF PLATES XXIII AND XXIV.

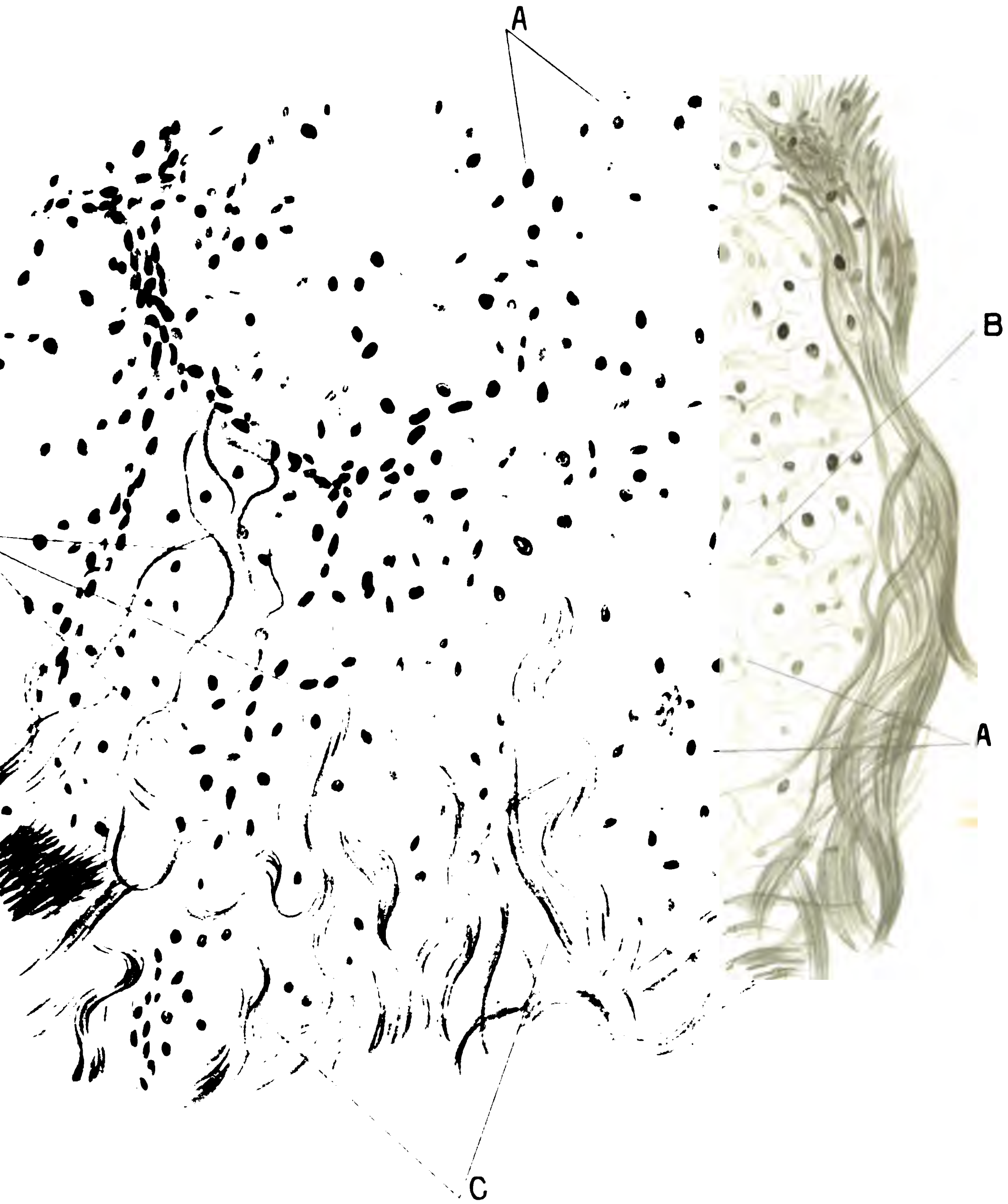
PLATE XXIII.

Shows size and appearance of normal fat cells from subcutaneous fat from back of neck of a normal pig aged one year. Drawn with camera lucida. Ocular 4, objective C (Zeiss).

PLATE XXIV.

Fat from back of neck of Pig 1, one year after commencement of diet consisting of milk from which fat was largely removed. A, fat cells which have undergone serous atrophy. Osmic acid does not stain the contents of these cells. The intercellular matrix is not represented. B, capillaries, some of them with blind ends. C, bundles of elastic fibres. Drawn with camera lucida. Ocular 4, objective C, Zeiss.




A
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STUDIES ON TRICHINOSIS, WITH ESPECIAL REFERENCE TO THE INCREASE OF THE EOSINOPHILIC CELLS IN THE BLOOD AND MUSCLE, THE ORIGIN OF THESE CELLS AND THEIR DIAGNOSTIC IMPORTANCE.

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PLATES XXV-XXVII.

INTRODUCTION.

Since the discovery by Zenker of the Trichina spiralis as a lethal agent in the human being, and the placing of the disease trichinosis or trichiniasis on a firm clinical basis, the subject has been studied with great care from many points of view. Indeed, the fact that with the study of this affection—its ætiology, symptomatology, pathology and prophylaxis—such names as those of Virchow (70), Leuckart (40), Cohnheim (13) and Zenker (74) are intimately connected, vouches for the completeness of our knowledge regarding many of its features.

Modern methods, however, have given rise to new possibilities in the line of more minute histological and pathological study, and it is to this fact and to the unusually favorable conditions surrounding the present cases that this contribution owes its origin.

The Trichina spiralis was discovered in the human cadaver in 1835 by Owen (53); it possessed, however, mainly zoological interest until, by the work of Zenker in 1860, a strong stimulus was given to the study of the trichina and of trichinosis. The various German epidemics (13, 57, 42) occurring since then have furnished abundant material for investigators, and as a result of their observations and experiments it is now known that the main source of infection in man is incompletely cooked pork, which in a certain percentage of instances contains encapsulated trichinæ in the muscle fibres.

After trichinotic flesh has been eaten the capsules are dissolved off by the gastric juice; the worms pass into the small intestine, where they reach their sexual maturity; copulation then takes place, and on the 6th or 7th day after the ingestion of the meat the first embryos appear. From the intestine the embryos pass to the muscular tissues. Some, especially Leuckart (40), regard this passage as taking place by an active boring of the embryos through the loose connective tissue; others (14, 2, 21) think that the distribution occurs chiefly through the vascular and lymphatic systems, a view which the recent work of Cerfontaine (14) and Askanazy (2) seems to favor. These observers assert that the female worms, after penetrating the villi, bring forth their young directly in the lymph spaces. After the embryos have reached the muscles, changes take place in them which will be described a little later.

CLINICAL HISTORY OF CASE I.

In the following case which was under observation in the medical clinic of the Johns Hopkins Hospital from March 3 until May 13, 1896, the blood was examined daily, with a determination of the number of leucocytes per cubic millimetre and a differential count of the various forms; frequent examinations of the urine and quantitative determinations of the uric acid, urea and total nitrogen were made, while on two different occasions small pieces of muscle were removed and subjected to careful microscopic examination.

Robert T., aged 23 years, an Englishman by birth, was admitted to the hospital on March 3, complaining of general pains. His past history was of no especial interest excepting that for the last three months he had been living the life of a tramp, with continual exposure to the weather and with insufficient nourishment.

Six weeks before entering the hospital he began to complain of general pains, as he expressed it, in the joints, bones and muscles. For the last two weeks he had been much worse, scarcely able to move about. On entrance he complained of a feeling of fulness in his head. No diarrhœa; no cough; no œdema at any time.

Physical examination showed the patient to be rather pale; tongue thickly coated; the temperature at time of admission was 102° F.; pulse 104; respirations 24. Examination of the thorax was negative, except for a soft systolic murmur heard all over the cardiac area; very faintly heard in the mid-axilla. Patient complained of general soreness in the limbs. Pressure on the muscles almost everywhere caused great pain, particularly in the right biceps. There was no tenderness apparent in the bones or in the skin. Spleen not palpable; no rose spots. Urine:

color normal; acid; 1028; albumin, trace; sediment, slight, showing mucous cylindroids, hyaline and granular casts. Diazo-reaction was not present.

Examination of the blood on March 5 showed 17,000 leucocytes with 37 per cent of eosinophiles. The absence of rose spots and of any palpable enlargement of the spleen, as well as the presence of a leucocytosis and the extraordinary increase in eosinophilic cells, rendered the diagnosis of typhoid fever, which was at first considered, improbable. The extreme muscular tenderness on pressure and on voluntary or passive motion, and the irregular temperature, suggested rather a myositis, and a diagnosis of trichinosis was made.

On further questioning the patient stated that six or seven weeks previously he began to have vague pains and heavy feelings in the muscles of his legs, and that he had repeatedly eaten raw or incompletely cooked pork during this period.

To confirm the diagnosis, on March 12 a small piece of muscle was removed under cocaine from the right biceps, where the pain was greatest at that time. A teased specimen of this showed, on microscopic examination, trichinæ, the majority actively motile, a few apparently non-motile; none were encapsulated, but many were surrounded by a finely granular material. During the remainder of the patient's stay in the hospital the symptoms gradually abated; the temperature, which had run a very irregular course, slowly descended to the normal point, and the sensitiveness of the muscles to pressure decreased. Œdema of the ankles appeared on two occasions in April after walking about the ward for several days, but both times quickly disappeared after rest in bed.

On May 13 the patient was discharged, the following note being made: "For from two to three weeks there has been no pain in the muscles; the stiffness has wholly disappeared and the patient feels perfectly well in every way, excepting that violent exertion is not readily borne."

Thus it will be seen that in many respects our case showed the classical picture of trichinosis. The pain in the muscles, aggravated by pressure or motion, the similarity of the early symptoms to those of typhoid fever, the œdema of the ankles after exercise, and the rather irregular fever, were characteristic. The absence of any history of gastro-intestinal symptoms, usually so marked, was probably due partly to the fact that this was not a case of extreme severity and partly to the incoherence of the patient on admission.

THE BLOOD.

Although it was known to the older pathologists that there were various kinds of white cells in the blood, it is only since the demonstration by Ehrlich (19) of their varied reactions to different dyes that we have possessed an adequate means of differentiating the various forms and of studying their relative proportions, one to another, in health and disease.

Morphologically, and by means of these tinctorial reactions, the leucocytes may, as is well known, be divided into five classes:

(1) Small mononuclear cells.
(2) Large mononuclear cells.
(3) Transitional cells.
(4) Polymorphonuclear neutrophilic cells.
(5) Eosinophilic cells.

In the first two divisions—the small and large mononuclear cells (the classification being determined by the fact that they are smaller or larger, respectively, than the ordinary polymorphonuclear neutrophiles) —the protoplasm shows no evidence of specific granulation and the nucleus is round or oval.

In the cells of the third division—the transitional—the nucleus becomes notched or indented and the protoplasm shows sometimes a slight tendency to neutrophilic granulation.

In the last two varieties, the polymorphonuclear neutrophiles and the eosinophiles, the granulations are well marked, in the former being fine and neutrophilic, in the latter coarse, highly refractive and acidophilic. The nucleus of the former is polymorphous; of the latter usually also polymorphous, although sometimes round or oval.

Many of the English school, notably Kanthack and Hardy (36), and Gulland (24), regard the so-called neutrophilic granulations as slightly acidophilic, and for this reason call the cells containing them the finely granular oxyphiles or acidophiles or eosinophiles. In the present work, however, the name polymorphonuclear neutrophiles (or cells with the ε granulation) will be used in describing them.

The percentage of these various forms in the blood differs quite markedly within normal limits. The usual proportions in the adult as given by Kanthack are:

Small mononuclears	15-25	per cent.
Large mononuclears and transitional forms	6	"
Polymorphonuclear neutrophiles	70-75	"
Eosinophiles	1-5	"

Concerning the proportion of the cells about which we are especially interested, the eosinophiles, various observers have given slightly different figures; Ehrlich (19*c*) regards it as from 2-4 per cent usually, although sometimes normally reaching 10 per cent; Hayem (31) as 7 per cent; Gabritschewsky (25), 1-3 per cent; Müller and Rieder (47*b*), 1-4 per cent; Canon (15), 1-3 per cent; v. Limbeck (41), 2-8 per cent; Uskov (quoted in 58), 6 per cent; Zappert (73), 0.67-11 per cent; while Weichselbaum (64) and Gollasch (26) each gives 5 per cent, including the transitional forms.

In this study the average number of leucocytes per cmm. has been taken to be 6500, of which 74 per cent (4810) should be polymorphonuclear neutrophiles, 18 per cent (1170) small mononuclears, 6 per cent (390) large mononuclears and transitionals, 2 per cent (130) eosinophiles.

Ever since the discovery of the specific cell granulations the eosinophilic cells have attracted the attention of many investigators who have studied the changes in the blood, especially in reference to the increase or decrease of this particular form in various pathological conditions. Much greater diagnostic significance than would seem to be warranted has, in many instances, been ascribed to this increase or decrease. Nevertheless, in certain diseases, chief among which are bronchial asthma and spleno-myelogenous leukæmia, a constant increase in these cells has been noted.

In the latter disease, as noted by Schwarze (60), Spilling (61), and Müller and Rieder (47*b*), the increase is more absolute than relative, owing to the enormous increase in the total number of leucocytes per cmm. In bronchial asthma an increase, both relative and absolute, has been described by Gollasch (26), Fink (20) and Gabritschewsky (25). In certain affections of the skin, especially pemphigus, prurigo, psoriasis and chronic eczema, a marked increase in eosinophiles has been noted by Canon (15) and Neusser (51). Kotschetkoff (37) describes an increase in scarlet fever, and Zappert (73) and Müller and Rieder (47*b*) in anchylostomiasis. Besides these, many isolated reports of an increase are to be found, although in many cases this has been too slight to be of significance. An increased percentage of eosinophiles has been noted in syphilis (4), malaria (1, 17, 27*a*), diseases of the genital organs and bones (51), mental diseases (51), during childhood (29), and after the injection of tuberculin (27*b*, 69, 5).

The largest percentages of eosinophiles hitherto reported have been 22.40 per cent in bronchial asthma (25), 33.02 per cent and 29.28 per cent in two cases of pemphigus, 27.9 per cent in anchylostomiasis (73), and 21.1 per cent in anterior poliomyelitis (47*b*). In a case of chronic eczema attending the Johns Hopkins Hospital, three different counts of the blood gave 22.6, 24 and 22.6 per cent of eosinophiles.

In the present case an examination of the blood on March 5 showed that the eosinophiles constituted 37 per cent of all the leucocytes—so striking an increase above the normal that from that time until the patient's discharge the blood was examined daily, and a determination of the number of leucocytes per cubic millimetre and a differential count of the various forms were made.

As a routine proceeding in making the differential estimate 500 leucocytes were counted daily, the stain employed being Ehrlich's triple stain (orange G., acid fuchsin and methylene green). In the case of the larger percentages an additional 500 stained in a different mixture (hæmatoxylin and eosin, methylene blue and eosin, Chenzinsky's solution, Huber's or Weichselbaum's modification of Ehrlich's triacid stain, Neusser's modification of the triple stain) were counted as a control.

The cover-glass preparations from which these counts were made were fixed either by Ehrlich's method of heating at 110° C. for from 1 to 1½ hours, or by Nikiforov's method of immersion in a mixture of equal parts of absolute alcohol and ether for two hours. The Zeiss mechanical stage was employed in studying the preparations.

In determining the number of leucocytes per cubic millimetre the Thoma-Zeiss hæmocytometer was used, the diluting agent being either Toison's mixture or an ⅓ per cent solution of acetic acid.

The red blood corpuscles, counted at various intervals during the disease, showed a gradual increase from 4,200,000 per cubic millimetre, three days after the patient's admission, to 4,900,000 per cubic millimetre on the day of his discharge.

When we consider the changes which the blood underwent during the two months of the patient's stay (see Table I, p. 322, and the Chart (Plate XXV)), some very interesting facts are brought out.

From their original percentage of 37, the eosinophiles gradually fell to between 10 and 15 per cent, where they remained for about two weeks, the number of leucocytes per cubic millimetre during this period ranging between 15,000 and 20,000. On March 26 the percentage of eosinophiles began to rise, this rise continuing steadily and gradually until April 23, when it reached 68.2 per cent; this means that *more than two-thirds of all the white blood cells were eosinophiles.* During this time an absolute increase in the leucocytes was also noted, the number per cubic millimetre averaging 27,500 between April 8 and April 22. From April 23 the percentage of eosinophiles decreased, and there was also a decrease in the total number of leucocytes, and on May 12, the day previous to the patient's discharge, 16.8 per cent and 11,000 were found respectively.

During the whole time the character of the eosinophiles was apparently perfectly normal—their nuclei were almost exclusively polymorphous, and they were slightly larger than the polymorphonuclear neutrophiles (Plate XXVI). As was to be expected, the percentage of these latter (always quite normal in character) varied inversely with that of the eosinophiles, reaching as high as 80.4 per cent and sinking as low as 6.6 per cent. The striking nature of this inverse relationship may be better appreciated if one studies the accompanying table and the chart (Plate XXV).

The small mononuclear leucocytes, scanty at first, soon increased to between 10 and 20 per cent, where they subsequently remained; in fact the percentage of both small and large mononuclear and transitional forms showed very slight fluctuations.

It is interesting to note that after about two weeks the percentage of small mononuclears kept fairly constant, being about that normally found in the blood. When we consider the very marked leucocytosis, this indicates that the total number of small mononuclears was much increased. When and how this increase takes place, however, must be left open to conjecture.

A few typical myelocytes were seen, averaging less than 0.3 per cent, probably no more than one would expect with so marked a leucocytosis.

TABLE I. CASE I.—SHOWING TOTAL NUMBER AND PERCENTAGE OF VARIOUS KINDS OF LEUCOCYTES.

DATE.	Leucocytes per cmm.	PERCENTAGE OF VARIOUS FORMS OF LEUCOCYTES.				NUMBER OF THE VARIOUS FORMS PER CMM.			
		P. M. Neut.	S. Monos.	L. M. and T.	Eos.	P. M. Neut.	S. Monos.	L. M. and T.	Eos.
March 5....	17000	55.0	3.0	5.0	37.0	9350	510	850	6300
6....	16500	50.0	5.0	7.0	38.0	8250	825	1120	6120
7....	17500	53.0	3.0	7.4	36.4	9275	525	1200	6300
8....	25300	60.9	1.9	5.9	31.4	15000	500	1500	7750
9....	22300	70.8	2.0	4.5	22.7	15300	440	900	5060
10....	16500	74.7	3.1	8.0	15.1	12000	480	1280	2400
11....	21167	75.1	2.8	2.4	18.6	15800	630	500	3700
12....	17500	77.8	4.4	3.0	15.1	13600	770	510	2625
13....	18833	77.6	2.1	3.7	16.6	13860	380	710	3000
14....	13800	74.6	4.3	4.0	14.9	10300	580	560	2100
15....	13750	74.0	7.6	3.2	13.6	10300	1000	420	1720
16....	13250	78.7	5.3	2.1	13.8	10400	680	270	1700
17....	16000	73.2	11.3	1.9	13.5	11700	1770	310	2160
18....	17750	77.4	10.9	1.7	10.0	13800	1970	300	1775
19....	20400	71.1	13.1	2.3	13.1	14200	2600	460	2600
20....	15700	76.5	10.1	2.2	11.2	12100	1570	340	1760
21....	16800	72.9	13.2	1.8	12.1	12400	1210	320	2040
22....	14000	79.2	10.8	1.7	8.3	11200	1540	240	1130
23....	16800	73.6	10.4	1.6	13.5	12400	1700	270	2200
24....	19600	74.6	11.2	1.8	11.2	14800	2200	360	2200
25....	17600	80.4	8.9	1.6	8.7	14400	1600	290	1560
26....	24000	69.2	12.7	2.0	15.4	16800	3120	480	3700
27....	20300	69.9	11.2	2.2	16.0	14000	2240	440	3200
28....	24100	68.8	10.1	2.4	18.9	16300	2400	580	4560
29....	20700	69.4	5.7	1.4	22.9	14000	1180	300	4600

TABLE I.—*Continued.*

DATE.	Leucocytes per cmm.	PERCENTAGE OF VARIOUS FORMS OF LEUCOCYTES.				NUMBER OF THE VARIOUS FORMS PER CMM.			
		P. M. Neut.	S. Monos.	L. M. and T.	Eos.	P. M. Neut.	S. Monos.	L. M. and T.	Eos.
March 30....	22300	60.7	9.6	3.0	26.0	13400	2200	670	5720
31....	22200	57.4	11.2	3.2	27.8	12600	2500	680	6160
April 1....	24800	60.7	7.9	3.4	27.8	14400	1920	840	6720
2....	23800	59.3	11.6	3.0	25.0	14100	2760	710	5950
3....	25200	53.3	13.3	2.8	30.0	13300	3500	700	7500
4....	23400	49.9	13.3	3.0	32.8	11700	3200	710	7590
5....	24300	48.8	13.2	4.0	33.8	10100	3150	960	7160
6....	24700	51.2	14.0	3.6	31.6	12700	3500	650	7900
7....	25100	48.4	14.5	2.5	33.7	12100	3600	625	8600
8....	29600	45.2	11.6	4.0	38.8	13500	3450	1180	11600
9....	28900	45.0	13.3	3.2	38.3	13000	3850	930	11000
10....	24500	45.8	12.2	3.8	38.0	11300	3100	900	9300
11....	29800	42.2	14.0	4.0	39.8	12600	4150	1180	11900
12....	28000	44.0	14.8	2.5	38.7	12300	4150	700	10800
13....	24200	42.0	11.8	1.4	44.2	10000	2880	340	10700
14....	33300	38.6	13.2	3.8	44.0	12800	4400	1300	14700
15....	28100	34.8	19.6	1.6	44.0	8600	5550	450	12300
16....	25400	30.4	16.8	3.0	49.6	7600	4200	750	12400
17....	27000	31.8	13.2	3.4	51.2	8600	3500	920	13700
18....	35700	27.2	14.4	3.8	54.6	9700	5140	360	19500
19....	29000	26.6	12.0	3.6	58.2	7700	3480	1040	16900
20....	19200	22.2	12.4	4.2	61.0	4200	2350	800	11600
21....	23000	16.6	16.0	3.8	63.6	3800	3680	870	14600
22....	26600	10.4	18.8	7.4	63.4	2700	4900	1950	16500
23....	17700	6.6	19.6	5.2	68.2	1170	3400	880	11070

TABLE I.—*Continued.*

DATE.	Leucocytes per cmm.	PERCENTAGE OF VARIOUS FORMS OF LEUCOCYTES.				NUMBER OF THE VARIOUS FORMS PER CMM.			
		P. M. Neut.	S. Monos.	L. M. and T.	Eos.	P. M. Neut.	S. Monos.	L. M. and T.	Eos.
April 24....	18800	7.2	23.4	4.4	64.8	1370	4400	840	12200
25....	15600	8.8	19.2	7.2	64.8	1370	2990	1020	10400
26....	17000	12.4	14.2	6.0	67.4	2100	2410	1020	11400
27....	12000	20.0	8.6	7.4	64.0	2400	1030	890	7700
28....	11100	18.2	17.6	7.2	57.0	2020	1950	800	6330
29....	12800	19.4	19.4	6.8	54.4	2470	2480	870	6990
30....	13200	20.8	17.2	8.2	53.8	2750	2270	1080	7020
May 1....	11000	21.8	16.0	12.0	50.2	2400	1760	1320	5520
2....	8900	27.4	17.8	8.4	46.4	2440	1580	740	5160
3....	10700	36.8	15.0	6.8	41.4	3940	1600	710	4430
4....	10700	34.8	20.8	6.4	38.0	3720	2120	680	4070
5....	11000	45.6	19.0	6.6	28.6	5020	2090	710	3150
6...	10300	41.0	20.2	4.8	33.6	4220	2080	490	3460
7....	12300	50.8	20.2	6.0	23.0	6250	2480	740	2830
8....	11500	54.0	18.0	4.0	24.0	6210	2070	460	2760
9....	11500	59.8	17.6	6.2	16.4	6850	2020	710	1890
10....	15700	51.0	12.8	5.6	20.4	7990	2010	880	3200
11....	13500	62.4	14.2	4.8	18.8	8420	1920	650	3540
12....	11000	64.0	14.4	4.8	16.8	7040	1580	530	1850

If one estimate the total number of these various forms per cubic millimetre (see Table I, p. 322) by multiplying the percentage of the individual variety by the total number of leucocytes per cmm., it will be found that, for a period of two weeks, the polymorphonuclear neutrophiles were *absolutely decreased* in amount—a very striking fact when one considers the marked leucocytosis. This tends to show even more clearly the remarkable relationship which exists between the neutrophiles and the eosinophiles, the latter cells being greatly increased in number during the same period.

Plate XXVI shows a typical microscopic field of the blood of Case I. The three most striking points furnished by the study of the blood are:

(1) The remarkable increase in the number of the eosinophiles, which amounted to 68.2 per cent of all the leucocytes present, 35 per cent more than has ever hitherto been reported.

(2) The coincident diminution in the number of the neutrophiles, these cells and the eosinophiles being at all times in inverse proportion.

(3) The marked leucocytosis.

The presence of such great quantities of eosinophiles in the blood in this case is a point of especial interest. If further observation shows this change to be characteristic we shall be furnished with a symptom of the greatest diagnostic value in this disease, and one which, perhaps, may help to clear up some of the cases which are regarded *intra vitam* as rheumatic in nature, the true diagnosis being revealed only years afterwards upon the autopsy table.

As an association has for a long time been noted between the eosinophiles and the Charcot-Leyden crystals, occurring so commonly in the spleen and marrow in leukæmia (52*b*) and in the sputum in bronchial asthma (20, 45, 46, 54), it was regarded as interesting to see whether from the blood in this case, containing as it did such large quantities of eosinophiles, the crystals might be obtained.

For this purpose blood was withdrawn on nine occasions, placed sometimes in sterilized, at other times in unsterilized vessels, some kept in the thermostat at 37.5° C., others at room temperature; but, although repeated and careful microscopical examinations were made at frequent intervals during a period of several weeks, in none of the specimens were any Charcot-Leyden crystals to be found.

This completely harmonizes with the conclusions reached by H. F. Müller (47*a*), who carried on investigations of a like nature upon the contents of fresh pemphigus vesicles, which also contain great quantities of eosinophilic cells. Thus it seems highly probable that the Charcot-Leyden crystals are, at least, not *direct* crystallization products of the eosinophiles and that something besides the presence of these cells is necessary for their formation.

THE URINE.

The urine, as has been mentioned, showed a trace of albumin, hyaline and finely granular casts and a small number of pus cells. No sugar was found. Ehrlich's diazo-reaction was never present.

A systematic quantitative study of the nitrogenous elements of the urine was carried on chiefly in connection with the ideas of Horbaczewski (30). This observer, as is well known, believes that the uric acid excreted by the animal organism is derived from the destruction of nuclein-holding material and mainly from the destruction of leucocytes.

This view is based on the following assumptions:

(1) That outside the body we may obtain uric acid from nuclein-holding substances.

(2) That in leukæmia and leucocytosis, where there is a greater destruction of leucocytes, there is a distinct increase in the uric acid excretion.

(3) That in many pathological conditions where nuclein-holding substances are destroyed, as in fevers, conditions of inanition, phosphorus-poisoning and cachexia, the increase in uric acid is also noted.

(4) That in the experimental leucopenias, produced by quinine and atropine, a diminution in the uric acid excretion may be shown.

The quantitative work was carried on in this case mainly with a view to control the second of the above statements, that is that a parallelism exists between the extent of the leucocytosis and the amount of uric acid excreted, a parallelism which has, however, been denied by Mareš (48), who regards the uric acid as a by-product in leucocyte formation.

To determine this question in the present case the total quantity of uric acid excreted during the 24 hours was determined on 23 different occasions; on four of these days the total nitrogen and on four others the total urea per 24 hours was also estimated.

The uric acid was determined by the Hopkins method—precipitation of the uric acid from 100 cc. of the urine by saturation with ammonium chloride, filtration, solution of the uric acid, reprecipitation with strong hydrochloric acid and final titration with potassium permanganate. The total nitrogen was estimated by the Kjeldahl method, *i. e.* conversion of all the nitrogen into ammonia and titration with a standard solution of oxalic acid. The urea was determined by oxidation with sodium hypobromite, and measurement of the nitrogen given off.

TABLE II. CASE I.—URINE CHART.

DATE.	TOTAL QUANTITY PER 24 HOURS OF			RATIO OF	
	Uric Acid.	Urea.	Total Nitrogen.	Uric Acid : Urea.	Nitrogen in Uric Acid : Total Nitrogen.
March 15....	.2423 gram.				
16....	.2092 "				
17....	.5722 "				
18....	.7800 "				
19....	.8180 "				
20....	.3869 "				
21....	.4053 "				
22....	.2088 "				
23....	.7935 "				
24....	.3119 "				
25....	.4579 "				
26....	.4542 "				
27....	.1975 "				
28....	.5882 "				
April 3....	.3685 "		7.9497 grams.		1 : 64.7
4....	.2829 "		7.2300 "		1 : 76.6
5....	.2753 "		4.6725 "		1 : 50.9
6....	.3619 "		4.8027 "		1 : 39.8
7....	.3971 "				
24....	.2905 "	10.79 grams.		1 : 37.1	
28....	.3716 "	19.22 "		1 : 51.7	
29....	.4893 "	29.11 "		1 : 59.4	
30....	.2946 "	17.21 "		1 : 58.4	

By examination of Table II it will be seen that on no occasion did the total uric acid excretion, the ratio of uric acid to urea excretion, or the ratio of nitrogen in the uric acid to the total nitrogen in the urine, rise to any extent above the limits usually regarded as normal.

The normal quantity of uric acid and the normal ratio of this to the urea is given somewhat differently by different authors; by Hammarsten (32) as 0.7 gramme and 1:50-70; by Halliburton (33), 0.55 gramme and 1:60; by Vierordt (71), 0.67 gramme and 1:45; and by v. Jaksch (35), 0.21 gramme and 1:60 respectively. Hammarsten (32) gives the normal ratio of nitrogen excreted in the form of uric acid to the total nitrogen as 1:69.3.

Thus the results obtained in this case show such a slight, if in fact any, relative or absolute increase in the uric acid excretion that they speak against the views of Horbaczewski; for if this author's results are universally correct we should expect a decided increase—absolute or at least relative—in the uric acid excretion in a case where the leucocytes were as markedly increased as they were in our case. It is an interesting fact, however, that here the eosinophiles and not the neutrophiles, as is usually the case in a marked leucocytosis, were the elements especially increased.

THE MUSCLES.

The changes in the muscles in trichinosis have, of course, been repeatedly and carefully studied; in the early sixties by Virchow (70), Zenker (74), Fiedler (21), Cohnheim (13) and many others; more recently by Soudakewitch (62), Lewin (43), Geisse (28) and Finger (22). All of these observers describe the presence of the parasites in the primitive muscle bundles and the granular degeneration which takes place about them; they further speak of the great proliferation of the muscle nuclei, so that in some places, especially in the region of the invaded fibre, there may be great masses or clumps of nuclei situated in undifferentiated sarcoplasm. In other places swelling and degeneration of the nuclei have been described. The method of formation of the capsule or cyst wall which surrounds the parasite after it has been in the muscle a certain length of time is still undecided. Many ideas have been held. It has been suggested that it arises from a thickening of the sarcolemma (70*a*, *b*, *d*, 6); from the interfascicular connective tissue (16); from the finely granular degenerative detritus (74*a*); or partly from secretion from the worm itself and partly from inflammatory changes in the surrounding connective tissue (7). The more modern observers have described further changes not mentioned by the older pathologists.

Finger (22) notes the presence of great numbers of small round cells about the vessels and capsules and in the interfibrillar connective tissue, and the splitting up of the contents of the sarcolemma sheaths into small irregular bits, in most of which the cross striation has been lost.

Soudakewitch (62), besides this breaking up of the muscle fibre into little bits, notes also the presence of great numbers of leucocytes which he regards as phagocytes of the degenerated fibres. This phagocytic action is also shared by elements which he regards as muscle-cells, and which arise by the separation from the rest of the muscle fibre of the nucleus with some of the muscle substance about it. These structures appear to maintain for a while an independent existence, but soon undergo destruction.

Lewin (43) has devoted himself more especially to the cell changes. He describes the proliferation and great hypertrophy of the muscle nuclei in the fibres containing trichinæ, with a degeneration of many of these nuclei as shown by the affinity of the nucleoli for the acid stain; the splitting up of the muscle fibre into separate cells; the formation of separate cells of the kind regarded by Soudakewitch as phagocytic muscle cells, and the local œdema of many of the fibres.

The portions of muscle removed in this case, both of which came from the right biceps, were hardened immediately, the specimen of March 12 in alcohol, that taken two weeks later in bichloride of mercury. On microscopic examination many of the above described changes are to be noted (Plate XXVII).

In the first specimen the reaction is especially marked in a few foci; in some places the fibre containing the parasite has become converted into a finely granular, faintly staining material, containing many large, swollen nuclei—the proliferated muscle nuclei—the nucleoli here taking the usual deep blue hæmatoxylin stain. The sarcolemma shows slight thickening, but there is no very marked heaping up of cells outside of it except in a very few places. Elsewhere absolutely no change in the muscle fibre is to be seen, excepting a small layer not thicker than the diameter of a red blood corpuscle, surrounding the parasite, which is very small; in fact, the whole picture points conclusively to a very recent infection.

In many of the fibres not containing parasites marked nuclear proliferation is seen; in some this is found in conjunction with granular degeneration of the muscle substance, in others with no apparent changes; about the nuclei, especially of the former, a distinct but rather small vacuole may be made out, a sign, probably, of lowered vitality of the nucleus.

A peculiar fragmentation of the muscle is sometimes to be noted. At times it takes place in the direction of the long axis of the fibres, which break up into separate fibrillæ or bunches of fibrillæ; at other times it occurs in an irregular manner. In a few places a peculiarly interesting picture is observed, the fragmentation being transverse and the axis of the proliferated nuclei running also in a transverse direction, thus splitting the muscle into distinct disks. Whether in this latter mode of fragmentation the division had occurred at the membrane of Krause or the plane of Hensen could not be made out, owing to the obscuring of the field by the great number of proliferated muscle nuclei.

Throughout the specimens there are to be seen cells whose protoplasm shows a marked affinity for the eosin stain. These cells contain one nucleus or sometimes two or three, generally of rather large size. The fact that some of these elements are still in part connected with the muscle fibre, and that in the protoplasm of some there are still evidences of striation, points at once to their origin by the separation of a certain amount of muscle substance, containing one or several nuclei from the primitive muscle bundle.

These cells, together with bits of degenerating muscle, some pale and granular, others taking on a deep eosin stain, but both showing a total or almost complete loss of striation, and many pale and swollen muscle nuclei, are seen in quite large numbers in certain scattered foci of degeneration present in the muscle.

What is of especial interest, however, is that all through the muscle, between and within the fibres, but especially in those areas made up largely of cells and debris, many polymorphonuclear cells are seen; some of these are ordinary pus cells, neutrophiles; others show large red refractive acidophilic granules in the cell body, *i. e.* they are eosinophiles; in some again the protoplasm, though finely granular, shows a distinct affinity for the eosin stain; in other words, the character of the cells appears to be half-way between that of the neutrophiles and that of the eosinophiles.

These cells would appear to be phagocytic in nature, for they are especially abundant in the more degenerated areas, and in many places are seen in little bays or inlets or lakes in the degenerating muscle, as if gnawing it away. It was thought at first that perhaps these cells might ingest bodily the little rod-shaped bits or fragments which are found especially at the ends of the degenerating muscle fibres and which with the hæmatoxylin and eosin stain show considerable resemblance to the granules in the eosinophilic cells; but staining with other double stains and with the triple stain showed that this supposition is hardly tenable, since the staining properties of the two are markedly different. The degenerated substance if taken up by the leucocytes must undergo a rapid disintegration within the cell body or possibly may be taken up in a soluble form.

In the second specimen, taken two weeks later, more advanced stages of the same processes are seen. The trichinæ are larger, and in some cases two or three parasites are found in the same cyst; about the cysts there is a marked increase in the number of nuclei; in some cases true giant cells are formed, a number of nuclei lying in a mass of undifferentiated protoplasm, probably sarcoplasm, while throughout the whole specimen the nuclear proliferation is more marked. The transverse splitting of the fibres into disks and the transverse proliferation of the contained nuclei is more marked than in the preceding specimen, as well as the longitudinal and irregular breaking up of the fibres.

The vacuoles about the proliferating and degenerating nuclei are more marked; the muscle cells, mentioned above, show less affinity for the eosin stain and in some cases give evidence of granular degeneration; besides the slight thickening of the sarcolemma there is no distinct capsule about the parasites. Of the polymorphonuclear cells, the eosinophiles are much more common, the neutrophiles much less numerous; the former are present everywhere, but are found especially in the scattered foci of more marked degeneration and about the ends of degenerating fibres (Plate XXVII).

That these were typical eosinophiles was shown not only by the character and size of their granules, but also by the fact that they showed the typical staining properties with acid fuchsin, orange G., picrate of ammonium and other acid stains, and the Biondi-Haidenhain triple stain for tissues as well as with the ordinary hæmatoxylin and eosin stain (Plate XXVII).

In the interfibrillary connective tissue there was a slight proliferation of the cells, while the changes in the interfascicular connective tissue were barely evident. In the blood-vessels in the latter, counts of the white blood cells showed that the neutrophiles and eosinophiles were present in about the same ratio (never differing more than 3 or 4 per cent) as in the blood count of the corresponding day, thus suggesting that the eosinophiles were not attracted to the part to a greater extent than the neutrophiles.

The presence of such great numbers of eosinophiles was so striking that two specimens of trichinotic muscle, found in the Pathological Museum of the Johns Hopkins Hospital, were examined to see whether like changes were present.

In the first, a case of carcinoma mammæ in which trichinæ were found in the pectoral muscle, the capsules and parasites were for the most part calcified, although some showed fatty and fibrous change; in the muscle no especial changes were noted. This, of course, was a case of old standing, discovered by chance, probably years after the original infection.

The second specimen, fortunately, was from a case of acute trichinosis which had caused the death of the patient, a New York butcher, six weeks after the onset of the disease. Here the muscle is studded with cysts, each containing from one to six trichinæ, while about these, and in fact all through the muscle, great quantities of nuclei are seen, about many of which there are distinct vacuoles; in some cases the nucleoli take the eosin stain. The muscle cells, mentioned before, here show still further degeneration; the protoplasm is granular and takes a yellowish color, while the nuclei stain very slightly with hæmatoxylin. But what is most interesting is that here again numbers of typical eosinophiles are to be seen scattered everywhere throughout the specimen, but congregated more especially in the areas of more marked degeneration, side by side with the degenerating bits of muscle and the muscle cells.

Thus these two cases, both acute and both studied shortly after the infection, besides the marked changes in the muscles, characterized by the great proliferation of nuclei, the formation of muscle cells and their subsequent degeneration, the formation of distinct vacuoles about the proliferating and degenerating muscle nuclei, and the granular degeneration of many of the fibres, show an interesting and hitherto undescribed condition, namely, the presence of large numbers of eosinophilic cells. This fact, coupled with their coincident marked increase in the circulating blood, suggests one way at least in which these cells may arise in the body.

THE INCREASE IN THE EOSINOPHILIC CELLS.

Concerning the nature of the eosinophilic granules and the origin of the cells containing them, many widely divergent views have been held, some founded entirely upon staining reactions, others built on a somewhat firmer basis, all more or less hypothetical, some very far-fetched and bizarre.

Until the introduction of the Ehrlich stains, these coarse, refractive granules were regarded as fat droplets by Wharton Jones (34), who first described them in 1846, by Förster (23) and Biesiadecki (8), who saw them in the blood in leukæmia, and by Neumann (52), Bizzozero (9), Budge (10) and Mosler (49), who noted them in the bone-marrow in the same disease. The cells containing these granules are the coarsely granular leucocytes of Max Schultze (63).

The work of Ehrlich (19) and Schwarze (60) showed, however, that these large refractive granules are not fat droplets, but bodies possessing a chemical nature not clearly understood. Ehrlich first demonstrated the affinity of this granulation for the acid coloring matters. These eosinophilic granules, as they are now generally termed, are regarded by him and his school as a sort of nutritive reserve, built up by the cell for its own use or for excretion. Thus, according to Schwarze (60), they resemble somewhat the aleuron granules or crystalloids of plants in being the products of a specific cell activity, and are probably not proteid in nature. Zappert (73) and Przewoski (56), however, insist that these granules are of the nature of proteid, the latter showing that their solubilities and chemical reactions correspond in many respects to this form of matter.

Many investigators, among whom may be mentioned Pouchet (55), Bannwarth (11) and Przewoski (56), basing their arguments upon the similarity in the reaction of these granules towards the acid aniline dyes, have regarded them as related to or identical with hæmoglobin, though Ehrlich (19) and Schwarze (60) deny this and show that there are quite marked tinctorial differences between the eosinophilic granules and hæmoglobin.

The demonstration of the presence of iron in these granules by Barker (12) is of especial interest in this connection.

Sherrington (65), who states that he demonstrated phosphorus in them by the Lilienfeld-Monti (44) method, regards them as a form of nucleo-albumin, while Kanthack and Hardy (36) consider them to be secretory products containing a zymogen, giving rise to alexines or other substances of great activity in phagocytosis and in conditions of inflammation and intoxication.

In all the above views the granules are regarded as paraplastic in nature. Gulland (24), on the other hand, holds that they are plasmatic; or, in other words, that they represent an altered condition of the microsomes and are connected by a mitoma.

As a consequence of this diversity of opinion regarding the nature of the granules, the mode of origin of the cells is still an unsolved problem. The view most generally held is that they are derived from the polymorphonuclear neutrophiles by a kind of ripening process, a view which was advanced by Max Schultze (63) and which is now held by Ehrlich (19), Gulland (24), Zappert (73) and many others. Müller and Rieder (47*b*, *c*) and van der Stricht (66) think that, besides this mode of formation, some eosinophiles are derived by mitosis from pre-existing cells of the same kind. Rénaut (59), Denys (18) and Arnold (3) describe amitotic division of these elements.

Max Schultze (63) and Müller and Rieder (47*b*, *c*) hold that this change takes place mainly in the circulating blood; according to Ehrlich (19) the bone-marrow is the only place where this transition occurs; while van der Stricht (66) and Zappert (73) think that blood and hæmatopoietic organs both play a part in this process.

Kanthack and Hardy (36) regard these elements as wandering cells whose home is normally the connective tissue; Saxer (68) thinks that all leucocytes are derived from pre-existing primary wandering cells, while Gollasch (26), Neusser (51) and Weiss (72) believe that they are derived directly from the tissue elements.

Of those who think they can trace a relationship between eosinophiles and the red blood corpuscles, Przewoski (56) regards the eosinophiles as incompletely developed erythrocytes, *i. e.* derived from hæmatoblasts, while Sacharoff (67) believes that they are formed through the engulfing by leucocytes of nucleoli cast out by hæmatoblasts (nucleated red corpuscles) at the time when the latter are converted into erythrocytes.

The study of the blood and the tissues of our cases of trichinosis furnishes us with important evidence bearing upon the origin of the eosinophilic cells. The constant inverse relationship existing between eosinophiles and neutrophiles, the increase of the former being invariably coupled with a coincident decrease of the latter, so that the neutrophiles on some occasions were absolutely subnormal in number notwithstanding the marked leucocytosis, as well as the similarity in size and the character of the nuclei of the two varieties of cells, both point strongly toward some very close connection between the two forms. Indeed, the striking and constant inverse relationship between the neutrophiles and eosinophiles would appear to be strong evidence in favor of the view that the latter cells arise, in some instances at least, by direct transformation of the former. There is much also to suggest that this transformation may have occurred here in the muscles. The presence in the first piece of muscle of many neutrophiles, together with many typical eosinophiles and cells which might be regarded as transitional forms between the two, and the finding in the second specimen two weeks later of a greatly increased number of eosinophiles, harmonize with this idea, especially when we consider that *on both of these occasions the ratio of the neutrophiles to the eosinophiles in the peripheral blood and in the vessels of the interfascicular connective tissue was between four and five to one*, a very much larger ratio than that which exists between these cells in the muscle.

How such a transformation might take place one can only conjecture. One may imagine that the trichinæ on reaching the muscle act as a strong irritant or poison, giving rise to many tissue changes, and probably producing either primarily or secondarily chemotactic substances which attract large numbers of leucocytes to the muscles, causing a marked leucocytosis, especially in the more degenerated portions. In these regions the polymorphonuclear neutrophiles wandering out of the vessels act as phagocytes upon the degenerating or degenerated bits of muscle. It is in these phagocytes apparently that the change in the character of the granules takes place.

By this it is not meant that the eosinophilic granules are bits of ingested material, but that they are elaborated by the cell itself. May not this result perhaps be due to the ingestion of some material originating from the degeneration of the muscle fibres, which may bring about some essential change in the character and chemismus of the cell?

Finally, we may well suppose that these cells wander or are swept back into the general circulation, giving us the marked increase in eosinophiles. Such a theory would not seem unreasonable, particularly when we consider the amount of muscle tissue in the body. The fact that practically no atypical neutrophiles or eosinophiles, *i. e.* evidences of transition, were seen in the blood itself speaks against the probability that such a change may have taken place in the circulation. Again, these small polymorphonuclear cells are certainly very different in appearance from the large, often mononuclear eosinophiles which we find in the blood and know to come from the marrow in leukæmia.

It is interesting to note that during the course of this investigation an article appeared by Kischensky (39), in which he comes to the same conclusion regarding the origin of the eosinophiles in the sputum of asthmatics, in which these cells are present in very large quantities, namely, that they are derived from neutrophiles.

After the completion of the above work and the publication of a preliminary note thereon in the *Johns Hopkins Hospital Bulletin* of April, 1897, a second case entered the Johns Hopkins Hospital, which in almost all respects tended to confirm the results obtained in the preceding investigations.

CLINICAL HISTORY OF CASE II.

The patient, E. B., a sailor, age 29, white, of German nationality, was admitted, April 15, 1897, complaining of fever, chills, muscular pains and weakness.

His past history shows that he has always enjoyed very good health; he gives no history of rheumatism nor of œdema, and has been only a moderate drinker. During his voyages his ship has often stopped at Cuba, but the sailors have never been allowed to go on shore, and he said there had been no sickness whatever amongst the crew. It was later found, however, that two members had malarial fever of the æstivo-autumnal type.

The present sickness dates from the first week in April. The onset was quite insidious; loss of appetite and general muscular weakness were noted, with occasional heavy painful sensations in the epigastrium. Headache set in for the first time on Friday, April 9, and continued until admission, the pain being especially marked in the frontal region. There had been considerable pain in the muscles of the back and especially in the calves of the legs since April 11. The bowels were regular until April 15, when the patient had two fluid stools, but no tenesmus. On April 10 the patient noticed a rather sudden œdema of the eyelids and face, causing him much discomfort and some pain in opening and closing the lids. He had had pain in the facial muscles during mastication for the last three days, while on deep inspiration there had been a slight pain through the chest. There had been no epistaxis. On board the ship the patient ate mostly salted meats and had frequently eaten raw sausage, though never raw ham. He had had shaking chills at times.

The physical examination showed heart and lungs negative; good color of lips and mucous membranes; gums normal; slight pain and stiffness in the masseters on opening and closing the mouth, and slight puffiness of the eyelids. Pressure on larynx is slightly painful, but no pain on pressure of calf muscles. No pain in arms and thighs. Spleen is just palpable; abdomen is a little full but not tender, and has upon it one or two spots somewhat suggestive of rose spots. There is no glandular enlargement; no tenderness or nodes on the long bones.

The temperature at first was of an intermittent type, rising each evening to between 103° and 105.5° F. and sinking to normal by the following morning. After April 24 the temperature continued normal until the time of the patient's discharge.

The stools, which continued to be of fluid consistence for some time, on frequent examinations showed no parasites or ova of parasites.

The urine was negative. The examination of the blood was made very frequently and carefully on many occasions, but no malarial parasites were ever obtained, nor were they obtained from the spleen, which was aspirated for this purpose.

The blood, however, showed other marked changes, chief among which was a great increase of the eosinophile cells, reaching 42.8 per cent on one occasion, accompanied by a moderate leucocytosis. This very marked eosinophilia, coupled with other points in the clinical history of the case, but mainly the eosinophilic increase, suggested the possibility that the case might be one of trichinosis. On April 28 a small piece of muscle from the calf was removed, and the diagnosis was confirmed by the demonstration in it of young non-encapsulated trichinæ. These were not present in such large numbers as in Case I, as might have been expected from the fact that the present patient complained of much less pain and in general showed less marked symptoms.

The subsequent history of the case was, as in the previous instance, a gradual cessation of all the symptoms, a disappearance of the muscular pain and a slow decrease in the percentage of eosinophiles, there being 17.6 per cent when he left the hospital, still a considerable increase above the normal.

THE BLOOD.

The blood in this case showed in many respects the same features as in the previous one (Table III). Both eosinophilia and leucocytosis, however, were less marked, as one would naturally have expected, since we were here dealing with a much less severe infection, if one may judge from the much milder clinical symptoms and the discovery of fewer parasites in the portion of muscle removed. As the patient was under observation a much shorter time, the table is not so complete as that belonging to Case I. The eosinophiles, which on admission made up 42.8 per cent of the total leucocytes of the blood, gradually sank to between 14 and 17 per cent, while in the corresponding period the polymorphonuclear neutrophiles rose from 43.1 to between 58 and 67 per cent (see Table III).

The leucocytosis varied between 6,000 and 13,000 during the patient's stay in the hospital, averaging about 10,000 and not varying markedly. The small mononuclears showed at first a tendency to be much decreased, as in the previous case; but, as was also seen before, showed later only slight fluctuations.

TABLE III. CASE II.—BLOOD CHART.

Date.	Leucocytes per cmm.	Percentage of the Various Forms of Leucocytes.				Total Number of Various Forms per cmm.			
		P. M. Neut.	L. M. and T.	S. Monos.	Eos.	P. M. Neut.	S. Monos.	L. M. and T.	Eos.
April 15....	13000	43.1	6.5	1.4	42.8	5600	180	840	5560
16....	....	47.7	4.7	4.5	39.1	....	....	....	....
17....	....	52.0	2.8	7.6	37.6	....	....	....	....
18....	8000	....	....	....	....	....	....	....	....
19....	....	57.2	2.8	8.0	32.0	....	....	....	....
20....	8900	55.2	3.8	11.2	31.8	4900	1000	340	2830
21....	8700	....	....	....	....	....	....	....	....
22....	10700	58.0	2.7	11.7	27.7	6200	1250	290	2960
23....	6000	56.2	2.0	15.2	26.2	3370	910	120	1570
24....	11000	60.4	4.0	13.2	22.4	6640	1450	440	2460
25....	11000	57.2	4.2	18.6	20.0	6290	2050	460	2200
26....	9600	60.7	5.3	12.3	21.7	5830	1180	510	2080
27....	11300	63.0	5.0	17.0	14.7	7120	1920	560	1660
28....	12700	62.7	4.0	17.0	16.3	7960	2160	510	2070
29....	12000	67.0	3.25	15.25	14.0	8040	1830	390	1680
May 1....	10700	58.3	4.0	22.0	15.7	6240	2350	430	1680
3....	13000	64.3	4.0	16.0	15.7	8360	2080	520	2040
5....	12000	64.7	4.0	16.0	15.3	7760	1920	480	1840
7....	9300	62.8	4.0	16.4	16.8	6140	1530	370	1560
10....	10700	60.4	2.8	16.4	20.0	6460	1750	300	2140
12....	11000	60.4	3.3	16.6	19.6	6640	1820	360	2160
14....	12000	58.8	2.8	18.8	19.6	7060	2260	340	2350
17....	9000	58.8	3.6	20.0	17.6	5290	1800	320	1580

When one considers the morphological structure of the different white blood cells one is again struck by the fact that mononuclear eosinophiles are almost entirely wanting, the nucleus of these cells being almost exclusively polymorphous and resembling in every way that of the neutrophiles. Here also, as in Case I, extremely few myelocytes were seen (averaging 0.03 per cent), and but few forms which could be regarded as transitional forms.

The number of red blood corpuscles per cmm. varied between 5,000,000 and 5,120,000.

THE MUSCLE.

The changes in the muscle in this case, although some of the features described before were lacking, resemble in many respects those seen in the first case. They were, however, by no means so well marked.

There is proliferation of the muscle nuclei, especially in the fibres, which show by their loss of staining properties a beginning degeneration. The number of degenerating fibres, however, is small, and these are entirely confined to the neighborhood of the trichinæ. The nuclei of the fibre containing the trichina are pale and greatly swollen, the nucleoli large and deeply staining; a few karyokinetic figures are seen.

Surrounding the large typically curved trichina in its muscle fibre, which shows granular degeneration, we have many small round cells, "muscle cells," muscle nuclei, polymorphonuclear cells (mostly neutrophilic, a few eosinophilic) and bits of muscles containing many swollen proliferated nuclei.

The striation of some of the degenerated fibres is entirely lacking; in others the peculiar granular degeneration noted in the first case is seen.

There are some eosinophiles scattered throughout the specimen, though not comparable in number to those noted in the first case; here more of the polymorphonuclear cells are neutrophiles.

There is no evidence of transition from neutrophiles to eosinophiles in this specimen. In fact the whole picture is one of a much less violent infection than in the first case. In all probability the trichinæ had been in the muscle fibre a longer time than in the first case, since when the bit of muscle was removed the number of eosinophiles per cmm. had fallen to a comparatively low point, nor did they rise again, a point which suggested, as did the clinical history, that the infection had almost run its course.

Recently, since the preceding pages were placed in the hands of the editor, a third case of trichinosis has come under our observation, which tends still further to establish our belief in the conclusions arrived at from the study of the first two cases.

In this, as in the second case, the discovery of a largely increased number of eosinophiles in the peripheral circulation led to the idea that the case was one of trichinosis, and an examination of an excised bit of muscle again proved the correctness of this idea.

Case III.—The patient, J. Y., was admitted to the hospital, December 21, 1897, complaining of headache, pain in the side, chilly sensations and swelling of the feet. Six months previously he had been treated for typhoid fever in the hospital and still gave the Widal reaction. Seven years previously he had had an attack of acute articular rheumatism.

The patient lived with his German employer and frequently partook of sausages and other forms of swine meat, often insufficiently cooked.

For five weeks he had been feeling unwell, complaining of frontal headaches and coughing spells; there had been no herpes, nose-bleed nor shaking chills. Five days before entering the hospital the patient had had an acute attack of diarrhœa, having 5 to 8 thin, green stools daily, associated with severe griping pains in the abdomen. Two days after the commencement of the diarrhœa he had noticed that his legs, feet, hands and arms seemed numb and cold. The joints were stiff and the limbs swollen and his attention was called by his employer to his swollen eyelids.

On admission the physical examination showed an absence of rose spots, spleen not palpable, puffiness of the eyelids, great tenderness of the muscles of the arms and of the calf, with swelling of the former and a bluish mottling of the skin of the limbs.

The temperature reached 103° for the first few days and then gradually fell to normal; shortly after this the pain in the limbs and the intestinal symptoms decreased, and on January 16, 1898, the patient was discharged, apparently quite well except for slight soreness of the right leg.

The blood (Table IV) on admission showed 45 per cent of eosinophiles, and this, from the study of the two previous cases, led to the diagnosis of trichinosis and the subsequent confirmation of that diagnosis by the removal of a portion of muscle and the discovery of trichinæ therein.

TABLE IV. CASE III.—BLOOD CHART.

Date.	Number per cmm.		Percentage of			
	Red blood corpuscles.	Leucocytes.	Polymorphonuclear neutrophiles.	Large mononuclears and transitional.	Small mononuclears.	Eosinophiles.
Dec. 22..	....	....	48.4	4.7	1.5	45.4
23..	....	17,000	52.7	3.6	3.1	40.4
27..	4,700,000	15,300	42.4	4.0	5.6	45.0
Jan. 3..	4,300,000	12,000	42.2	4.2	4.6	49.0
7..	....	14,700	31.6	4.4	19.0	44.6
10..	4,546,000	....	32.4	3.0	21.8	42.8
14..	....	13,700	35.2	4.0	23.4	37.2
22..	....	9,000	45.5	2.0	17.7	34.7

During the stay of the patient in the hospital the proportion of eosinophiles varied very slightly, reaching 49 per cent on January 3 and sinking to 34.7 per cent on January 22. As in the other cases, the nuclei of practically all these cells were polymorphous; yet, as before, no forms were seen in the blood suggesting transitional stages between neutrophiles and eosinophiles. At first there was a smaller percentage than normal of the small mononuclears, which, however, increased later up to practically the normal, a condition which was noted in both the preceding cases. On one occasion, and only one, two typical myelocytes were seen. The leucocytosis was not marked, being 17,000 on December 23 and 9,000 on January 22 (see Table IV).

As in the preceding cases, it was impossible to determine how long the eosinophilia lasted, as in all three cases the patients left town and were lost from observation.

The stools, although examined repeatedly, showed no intestinal trichinæ.

The piece of muscle removed during the first week of the patient's presence in the hospital showed in almost all respects the same changes noted in the two previous cases, except in this case the specimens of the parasite found in the muscle were younger, smaller and less abundant than in the first case. In the muscle fibre in which the trichinæ were found there was a complete loss of striation and a tendency to take on a faint bluish stain. In these fibres the proliferated and greatly swollen muscle nuclei with their deeply staining nucleoli were seen arranged circularly about the trichinæ lying in the finely granular, faintly staining, degenerated muscular substance.

Near the infested fibres were found degenerating areas made up of disintegrating bits of muscle fibre, muscle nuclei, small round cells, and polymorphonuclear cells, a great many of whose granules were eosinophilic. These eosinophilic cells were especially prevalent about the bits of degenerating muscle and were certainly as numerous as in the first case.

There was the peculiar granular degeneration of the muscle fibres noted before, and the proliferation of the muscle nuclei in the neighborhood of the parasite.

The trichinæ were young, only slightly curled up and of course non-encapsulated.

In severity, clinically, this case lies midway between the first and second cases, and this position is borne out by the number of parasites found in the muscle excised, by the leucocytosis and by the grade of eosinophilia.

SUMMARY.

To summarize, we have been able to demonstrate:

(1) In a case of acute trichinosis an extensive leucocytosis, with great absolute and relative increase in the number of eosinophilic cells in the blood, associated with a coincident decrease in the quantity of neutrophilic elements.

(2) From the examination of specimens of muscle removed during life, besides the peculiar degenerations of the muscle, a longitudinal

splitting of some of the fibres; a remarkable transverse splitting of others; a great proliferation of nuclei, about many of which vacuoles are seen; and large numbers of polymorphonuclear eosinophilic cells, which are especially prevalent in the more degenerated areas.

(3) In a second case (after death), besides similar changes in the muscle, large numbers of eosinophiles throughout the infested portion.

(4) In two other cases, during life, a great increase of the eosinophilic cells in the blood, with a coincident decrease of the polymorphonuclear neutrophiles, associated with leucocytosis, though of less extent than in the first case.

(5) In pieces of muscle removed in these last two cases changes in most respects similar to those cited in the first case, but of less degree.

(6) The similar character of the nuclei of the eosinophiles and the neutrophiles both in the blood and in the muscle, and the presence in the first case of certain cells which might be regarded as forms transitional between neutrophiles and eosinophiles, suggesting the possibility that the increase in the latter elements may, in these instances, take place in the muscles by direct transition from the neutrophiles.

CONCLUSIONS.

From these observations it is fair to conclude:

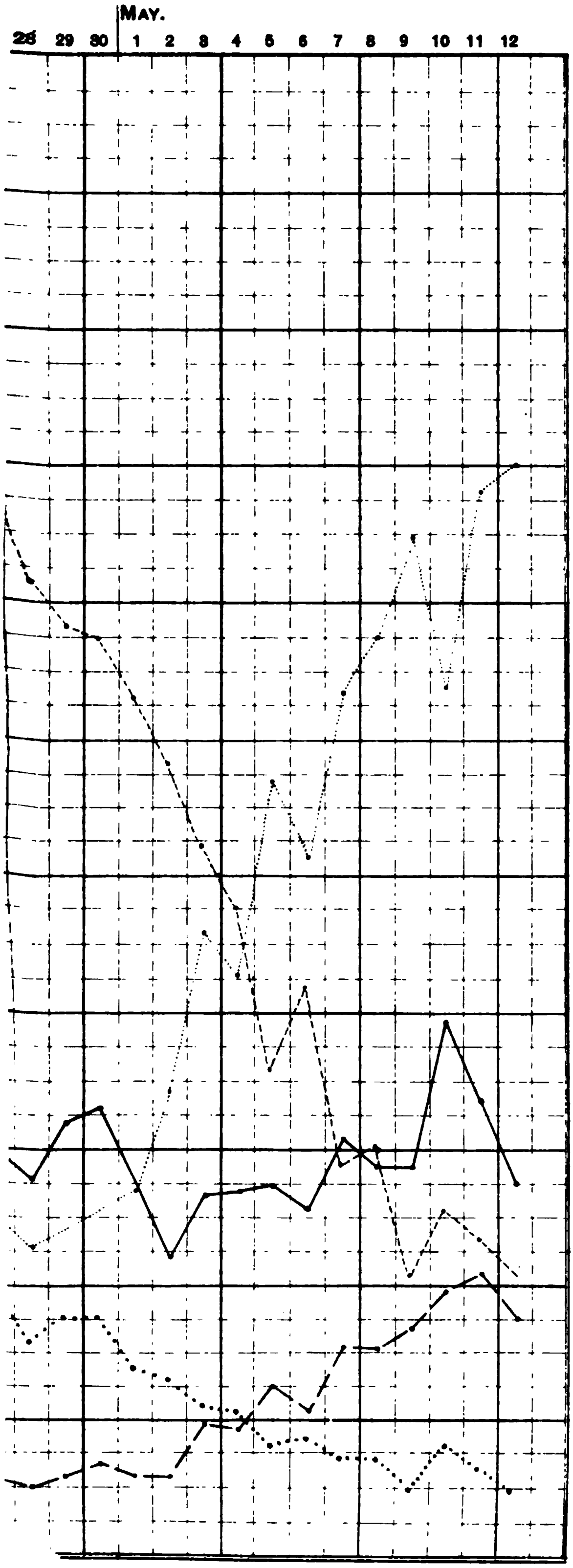
1st. That there is a marked increase in the percentage of eosinophilic cells in the blood in trichinosis.

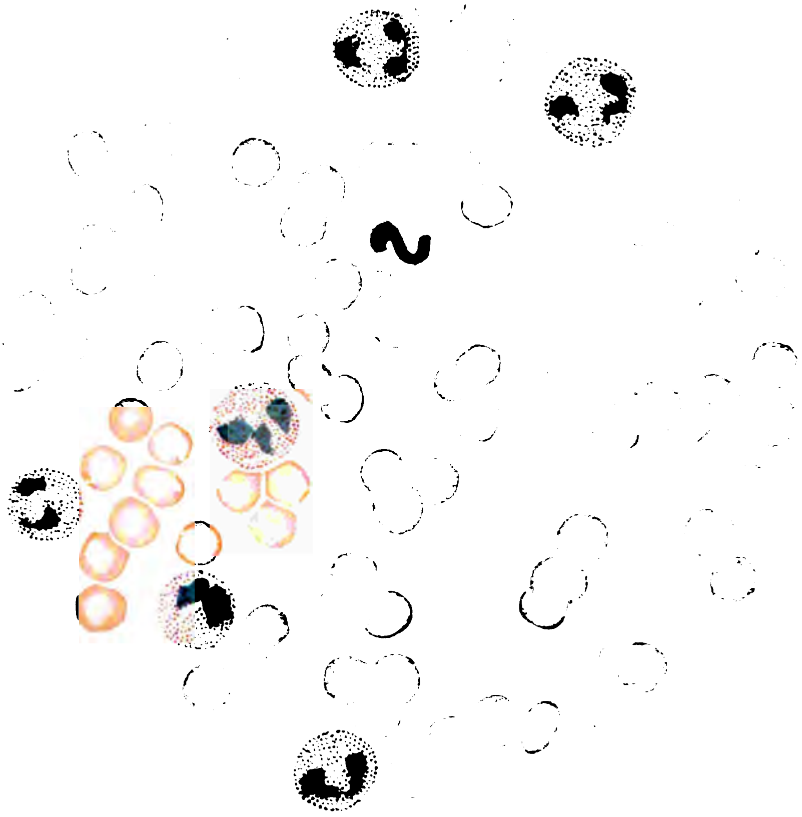
2nd. That this increase may be used as a diagnostic sign in this disease.

3rd. That this disease in its sporadic form is more common than has hitherto been supposed, as shown by the discovery of the three cases above described, within a comparatively short period, at the Johns Hopkins Hospital.

4th. That a systematic examination of the blood should be undertaken in cases with indefinite intestinal, muscular or articular symptoms, in the hope that in some, at least, of the hitherto doubtful cases a diagnosis may be reached.

In conclusion I wish to express to Professor William Osler my sincere appreciation of his kindness in giving me the opportunity of es and of Eosinophiles.





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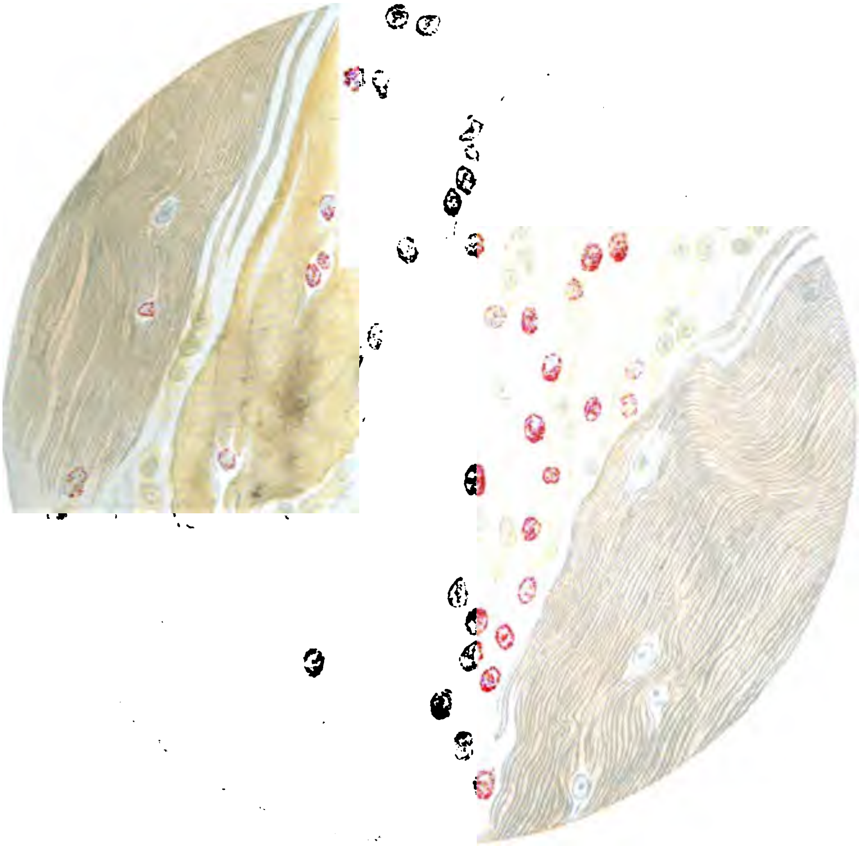
THE JOURNAL OF EXPERIMENTAL MEDICINE. VOL. III. PLATE XXVII.



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studying these cases, and to Dr. William Sidney Thayer my hearty thanks for his help during the course of this investigation.

DESCRIPTION OF PLATES XXV–XXVII.

PLATE XXV.

Chart showing graphically the total number and percentages of the various kinds of leucocytes.

PLATE XXVI.

From the blood of Case I, showing a typical field, containing six eosinophiles, with polymorphous nuclei, one polymorphonuclear neutrophile, one large mononuclear leucocyte and many erythrocytes.

PLATE XXVII.

Showing degenerating area in muscle in neighborhood of trichina, demonstrating beginning loss of striation in two fibres, complete loss in a third, and in the rest of the specimen, muscle nuclei, bits of degenerating muscle tissue and many polymorphonuclear eosinophiles. Haidenhain-Biondi triple stain.

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ON THE PRESENCE OF THE TYPHOID BACILLUS IN THE URINE.*

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The fact that the urine of individuals sick with typhoid fever may, at times, contain the specific organism of the disease has, during the past decade, occupied the attention of a considerable number of observers. The results obtained, and the conclusions drawn, are briefly as follows:

Bouchard,† in 65 cases of typhoid fever, found in the urine no bacteria and no albumin in 44. In 21 cases there were bacteria, and these were always associated with albumin. They were bacilli and were assumed to be typhoid organisms.

Hueppe ‡ succeeded in obtaining the typhoid bacillus from the urine in only one out of 18 cases.

Seitz § obtained positive results in 2 out of 7 cases. The urine of both positive cases showed albumin, and in one case also casts. The organisms, when found, were present in large numbers.

Konjajeff || found typhoid bacilli in the urine of 3 out of 20 cases. Albumin was present in only one of these positive cases. The organisms appeared generally in the later stages of the disease (third week), and were always in large numbers. He thinks that the presence of the bacilli is always associated with the so-called typhoid lymphomata in the kidney.

Karlinski ¶ examined the urine in 38 cases in which the symptoms were those of typhoid and where the specific bacilli had been cultivated from the stools. He also examined bacteriologically the bladders at 6 typhoid autopsies. Out of these 44 cases the typhoid bacillus was isolated in 21, and was always associated with albuminuria. When found

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* A partial report of investigations carried on under the provisions of the Dalton scholarship at the Massachusetts General Hospital.

† Bouchard, *Rev. de méd.*, i (1881), 671.

‡ Hueppe, *Fortsch. der Med.*, iv (1886), 448.

§ Seitz, Bakt. Studien zur Typhus-Aetiologie. München, 1886.

|| Konjajeff, Ref. in *Centralbl. f. Bakt.*, vi (1889), 672.

¶ Karlinski, *Prag. med. Woch.*, xv (1890), 437 and 452.

the bacillus was always in pure culture. K. claimed to have obtained a positive result as early as the third day of the disease.

H. Neumann * out of 48 cases obtained positive results in 11. He found the bacilli always in large numbers, but thought he probably failed to detect them when their number was small. In his experience albumin was not necessarily an accompaniment of the bacilli. Moreover, albumin was often present without bacilli. Furthermore, Neumann pointed out the fact that typhoid bacilli can reproduce themselves rapidly, even in an acid urine, and that the bladder, with its constant supply of fresh urine, offers a peculiarly favorable place for the multiplication of the organisms. The prognosis was not necessarily worse in consequence of the presence of the bacilli in the urine. Neumann made the important statement, also, that the typhoid organisms may persist a considerable time after the cessation of fever, and that the urine of typhoid convalescents ought, therefore, to be subject to very careful supervision. He thought the bacilli were finally removed mechanically. In some cases cocci were found, and these were supposed to indicate a complication.

Geisler,† from a review of the literature, concluded that typhoid bacilli could be isolated from the urine in about 26 per cent of the cases.

Borges ‡ obtained positive results in 2 out of 10 cases. In both cases there was albumin in the urine. B. thought that the albumin and the bacilli disappeared together. In one-half of the cases there was evidence of pathological change in the kidneys, whereas in only one-fifth of the cases were there bacilli.

Baart de la Faille § obtained typhoid bacilli in the urine of 4 out of 27 cases. In 4 other cases he found colon bacilli.

G. Blumer || found pus in the urine in 10 out of 60 cases, and this pus was always associated with bacteria. The colon bacillus was present 7 times, 6 times alone and once together with the typhoid bacillus. The typhoid bacillus was found twice, once with the colon bacillus and once alone. The staphylococcus pyogenes albus appeared in one case, and in another an unrecognized coccus was observed. Blumer thought the bacteria reached the bladder perhaps through the recto-vesical wall.

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* Neumann, *Berlin. klin. Woch.*, xxvii (1890), 121.

† Geisler, Ref. in *Centralbl. f. Bakt.*, xiii (1893), 767.

‡ Borges, Ueber die Durchlässigkeit der Typhusniere für den Bacillus typhi abdominalis. Inaug.-Diss., Würzburg, 1894.

§ Baart de la Faille, Inaug.-Diss., Utrecht, 1895. Ref. in *Centralbl. f. Bakt.*, xviii (1895), 337.

|| Blumer, *Johns Hopkins Hospital Reports*, v (1895), 327.

The pus in the urine he considered important only because of its possible association with the typhoid bacillus, and therefore recommended that the patients should be kept in the hospital till the pus had disappeared.

Wright * had the unusually good fortune to find typhoid bacilli in 6 out of 7 cases.

Besson † examined the urine of 33 typhoid patients and obtained typhoid bacilli in 6. In 12 urines containing only slight traces of albumin bacilli were found but once. In 12 cases with considerable amounts of albumin the bacilli were present in 5.

As we look over the writings of these observers we find that the following general facts were brought out by their work:

(1) The typhoid bacillus was demonstrated in the urine of typhoid patients in about 25 per cent of the cases.

(2) The bacilli, when demonstrated, were present nearly always in large numbers and, in the great majority of cases, were associated with albuminuria. On the other hand considerable amounts of albumin were often present in the urine without bacilli.

(3) The organisms appeared generally in the later stages of the disease, but, having appeared, they might persist for a considerable time into convalescence.

These conclusions, then, represent in the main the results obtained up to the year 1898.

Of course we must take into consideration the fact that in the early days of the literature relating to the typhoid bacillus the positive identification of this bacillus was much more uncertain than at present, and that confusion of the typhoid organism with other bacilli, especially those of the colon group, must have occasionally taken place. We must, therefore, view with some suspicion such results as those of Karlinski, ‡ who obtained positive results in 50 per cent of his cases, and that, too, in very early stages of the disease. On the other hand the results of Wright § were no less unusual (6 positive

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* Wright, *Lancet*, 1895, ii, 196.

† Besson, *Rev. de méd.*, xvii (1897), 405.

‡ Karlinski, loc. cit.

§ Wright, loc. cit.

cases out of 7), although, in this instance, the more important of the modern differential tests were used.

The following details of my own investigations and results will be found to confirm substantially the conclusions stated above.

The methods employed in the work were briefly these: In the case of male patients (by far the larger number) the glans penis was first thoroughly cleansed with corrosive sublimate solution 1:1000. The urine was then passed in two portions into sterilized test tubes. Only the second portion was used for bacteriological purposes. The female patients were catheterized with all possible antiseptic precautions, and in these cases also only the second part of the urine was used.

The urine obtained in this manner was then plated, with as little delay as possible, upon plain agar. Two plates were made for each specimen, one representing a single loop of urine and the other 1 cc. (approximately). After being left for 18 to 24 hours in the incubator the plates were examined under a low power of the microscope (Zeiss AA or Leitz No. 3).

It might be thought that difficulties similar to those encountered in making cultures from the stools might be met with also in the case of the urine, and that, therefore, a differential medium, such as that of Elsner, Capaldi or Hiss, would be necessary in this work. But it has been found that in nearly all cases we have to do with but a single organism (rarely two or three), so that ordinary media answer our purpose perfectly well. The colon colonies are, naturally, the ones most easily mistaken for the typhoid, but a little experience makes it possible to tell them apart.

In cases of doubt I have made use of the following procedure with great satisfaction as a preliminary test: If we examine closely the rent in a typhoid colony, which we make in the process of inoculation with the platinum needle, we can see oftentimes, even with the low power, a marked seething motion resembling very much that of a swarm of bees. This phenomenon is due, of course, simply to the setting free of the very motile bacilli, and becomes much more evident with a higher power of the microscope (Zeiss DD or Leitz No. 7). The amount of motility varies in different colonies, but it is very rare that one is not enabled by this procedure to say whether the organism is or is not actively motile. In my experience with typhoid urine and fæces, motile bacteria of other varieties have not been sufficiently numerous to invalidate the procedure as a preliminary test.

The cultures of cocci isolated during the work were examined only as to their shape and arrangement. The typhoid and colon cultures were subjected to the following differential tests:

(1) Hanging drop for size, shape and motility.

(2) Growth upon gelatin slant and in stab cultures.

(3) Growth upon the tube medium of Hiss[*] for the presence or absence of general clouding and gas production.

(4) Growth in peptone solution. Presence or absence of indol.

(5) Growth upon potato. Presence or absence of visible growth.

(6) Growth in the two test solutions of Proskauer and Capaldi.

(7) Growth in litmus-milk.

(8) Reaction to typhoid serum.[†]

Coming now to a presentation of the results of the investigation we find that 172 specimens of urine, representing 38 cases of typhoid fever, were examined. Of these 172 specimens 44, representing 9 cases, showed the presence of typhoid bacilli. In 2 cases, one positive and the other negative as far as the presence of the typhoid organism was concerned, the urine was examined first at autopsy (Cases II and XXI).

The following tables give the results in detail. The numbers refer to the day of the disease as nearly as it could be calculated from the history.[‡]

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[*] *Journal of Experimental Medicine*, ii (1897), 677.

[†] In carrying out this test I have always made use of the dried serum, or "serum paper," described by me in the *Centralbl. für Bakt.*, xxi (1897), 445. The paper is now 14 months old and shows no appreciable decrease in its agglutinating power. It is certainly a most convenient form for preserving the test for laboratory use, especially in localities where typhoid cases are scarce, and where, therefore, fluid serum is not always to be had.

[‡] I am indebted to the records of the Massachusetts General Hospital for my information as to the presence or absence of casts, and as to the clinical features of the cases.

TABLE I. CASES I TO IX.—POSITIVE CASES.

Number of case.	Sex.	Cultivations made from urine.	Typhoid bacilli found.	Albumin present.	Casts.	CLINICAL FEATURES.					Temp. normal.	Relapse.	Day of discharge.	REMARKS.
						Rose spots.	Delirium.	Hæmorrhage.	Bronchitis.	Cystitis.				
I	M	38.42.47.54. 59.63.67.70.72 78	38.42.47.54 59.63.67.70.72 78	38.42.54.59 63.67.70.78 always in faint traces.	8 = no casts. No record thereafter.	+	0	0	pneumonia 35	0	24	27 to 56	72	In relapse a pneumonia. Typhoid bacilli in sputum 44th, 47th, 49th days. Phlebitis 28th day.
II	M	34.	34.	5 = faint trace	15 = present.	+	+	#	+	0				On 33rd day symptoms of perforation. Operated upon. Died same night.
III	M	23.25.28.33 41	23.25.28.33 41	23.25.28 = faint traces. 41 = 0	3.19.29 = present.	+	0	0	0	0	27	0	44	During convalescence urine averaged 70 ozs. daily.
IV	F	33.38.43.53	33.38	33.38.43 = very faint traces 53 = 0	20.26.28 = present.	+	0	0	0	0	40	0	55	Catheterized upon 13th day.
V	M	20.30.36.40	36.40	30.36.40 = faint traces	10 = present.	0	#	+	+	very slight.				Died upon 43rd day.
VI	M	8.11.15.20.25 27.30.31.33 34.36.37.39 43.66	15.20.25.27 30.31.33.34 36	8.11.15.25 = a trace. 27.30.31.36.37 39.43.66 = faint trace.	8 = present. 30 = absent.	+	0	0	0	+	19	0	43	Catheterized on 8th and following days. Bladder irrigated with boric acid 2% on 30th and 33rd days. No effect on bacilli. On 36th day irrigation with corrosive sublimate 1:7000. No bacilli on 37th, 39th, 43rd, 66th days.
VII	M	12.15.17.24.28 33.40.49.59	24.33.40.49	12.15.17.24 = ⅛% 28.33.40.49 59 = large trace.	6.12.18.35 = present.	0	0	0	0	0	48	0	59	Marked post-febrile polyuria.
VIII	M	12.24.31.32 37.45	32.37.45	12.37.45 = faint trace.	30.38 = No casts found.	0	0	0	0	0	29	0	48	Furunculosis. Cultivation showed staphylococcus aureus in pure culture.
IX	M	38.43.46.52.58 65.72	38.43.46.52 58.65.72	14.38 = trace. 43.46.52.58 65.72 = faint trace.	14.31.52 = present.	+	0	0	0	0	35	38 to 58	67	Catheterized on the 14th and following days. Epididymitis on the 45th day. Urine during last week in hospital averaged 80 ozs. daily.

Number of case.	Sex.	Cultivations made from urine.	Bacteria found.	Albumin present.	Casts.	CLINICAL FEATURES.					Temp. normal.	Relapse.	Day of discharge.	REMARKS.
						Rose spots.	Delirium.	Hæmorrhage.	Bronchitis.	Cystitis.				
X	F	49.	0	49=large trace.	11.16=present.	0	+	0	+	0	27	42 to 58	60	
XI	M	24.32	0	24.32=0	0	0	0	0	0	0	30	0	43	
XII	M	18.23.26.31 40.54.65	0	18.23.26.31.40 54.65=0	0	+	0	0	0	0	28	46 to 58	79	
XIII	F	16.20.23 35.40.48	16.20.28.35=a diplococcus 40.48=colon bacillus.	8.16.23.46=faint trace. 25.35.40=a trace.	8.28.33=present.	+	+	0	0	+	29	0	62	Catheterized for a considerable time.
XIV	M	14.26	0	14.26=0	0	0	0	0	0	0	25	0	38	
XV	M	26.38.45 54.57	0	26.38.45.54.57=0	0	0	+	0	0	0	70	0	78	
XVI	M	15.29	0	15.29=0	0	+	0	0	0	0	24	24 to 31	53	Phlebitis on 39th day.
XVII	M	15.18.19.31 38	18.19.31=a streptococcus 38=colon bacillus.	15.18.19.31.38=0	0	0	0	0	0	0	30	0	46	
XVIII	M	24.45	0	24.45=0	0	0	0	0	0	0	20	0	48	
XIX	M	20.25.33 37.40	20.25.33.37.40=a diplococcus.	20.25.33.37.40=0	0	+	+	+	0	0	28	0	52	Pulse reached 147 at one time.
XX	M	24.31.39	0	8.24.31=0 39=ft. trace.	8=present.	0	+	0	+	0	28	0	40	
XXI	M	38	0	25=very faint trace.	25=present.	0	+	0	0	0			Died 38	Culture made from bladder at autopsy. A very severe case with high fever and pulse rate.
XXII	M	24.32.38	24.32.38=colon bacillus	24=faint trace.	0	+	0	0	0	+	26	0	44	Catheterized on 18th day.
XXIII	M	9.16.24.33	0	9.16.24.33=0	0	+	0	0	0	0	20	0	36	

TABLE II.—*Continued.*

Number of case.	Sex.	Cultivations made from urine.	Bacteria found.	Albumin present.	Casts.	CLINICAL FEATURES.					Temp. normal.	Relapse.	Day of discharge.	REMARKS.
						Rose spots.	Delirium.	Hæmorrhage.	Bronchitis.	Cystitis.				
XXIV	M	20.27	0	20.27=0	0	0	0	0	0	0	20	0	31	
XXV	M	10.21	0	10.21=0	0	0	0	0	0	0	21	0	34	
XXVI	M	9.14.22.29 36	9.14.22.29.36= colon bacillus.	9.14.22.29.36 =faint trace.	15=present.	0	0	0	0	+	30	0	46	Bladder operated on a year before entrance. Came in with cystitis.
XXVII	M	42.45	0	33.42.45=0	33=present.	0	0	0	0	0	35	+	46	Probably came in during a relapse.
XXVIII	M	18.23.27 34.41	18.23.27.34.41= a diplococcus. 41=0.	15.18.23.27 =faint trace. 34.41=0	15=present.	+	0	0	0	very sl.	38	0	60	
XXIX	M	18.22.25 27.33	0	18.22.25.27=0 33=faint trace.	0	+	0	0	0	0	28	0	47	
XXX	M	23.25.32	0	23.25=ft. trace. 32=0	21.28 present.	0	+	0	0	0	25	0	45	
XXXI	M	21.25.31.33 35.39.42.54	0	21.25.31.33.35.39 42.54=0	0	0	+	0	0	0	45	0	65	
XXXII	F	24.28.30 35.44.51 56	44.51.56= colon bacilli.	21=¼% 24.28.30 38.44.51.56 =large trace.	21=present.	0	+	0	0	sl.	56	0	72	Catheterized.
XXXIII	M	13.15.17.27	0	13.15.17.27=0	0	0	0	0	0	0	24	0	42	
XXXIV	M	25.36.51 53	0	25=faint trace. 36.51=0	20=present.	+	sl.	0	0	0	44	0	56	
XXXV	F	24	24= colon bacillus 25= colon bacillus (autopsy).	9=⅛%.	9=present.	+	+	+	+	0			25 Dead.	
XXXVI	M	9.15.20.34 49	0	9.15.20.34.49 =faint trace.	49=present.	+	0	0	0	0	37	0	52	
XXXVII	M	15.19.24 37.44	0	15.19=ft. trace. 24.37.44=0	23=present.	0	0	0	0	0	36	0	50	
XXXVIII	M	33.38.43 51.57	0	33.38=trace. 48=faint trace.	30=present.									

From an examination of the table of positive cases (I to IX) we see that, in 5 out of the 9, the bacilli were found on the first day of cultivation. How much earlier they may have been present it is, of course, impossible to say. The other 4 positive cases, although examined from a comparatively early period of the disease, showed no bacilli until the 15th, 24th, 32nd and 36th days respectively. The invasion of the urine by the typhoid organism seems to be, therefore, a late rather than an early occurrence. For this reason the examination of the urine bacteriologically for the purpose of diagnosis would seem to have but little value as compared with the specific serum test. All the cases under consideration showed at some time during the disease the so-called Widal serum reaction.

A more important point, and one to be very strongly emphasized, is the length of time that the bacilli may persist in the urine after its infection. Of the 9 positive cases 7 survived the disease and were discharged as well. In 5 of the 7 cases, however, the urine still contained, upon the day of discharge from the hospital, typhoid bacilli in undiminished numbers. This fact would be, perhaps, of less importance if each loop of urine harbored one or two bacilli, but when it is considered that in most cases a single loop contained many hundreds, or even thousands, of organisms, then the matter assumes an entirely different aspect, especially when viewed in its relation to disinfection and the public health. Modern sanitary regulations forbid that diphtheria patients in whose throats Klebs-Löffler bacilli persist shall be suffered to go abroad. Is it not just as important that there shall be a similar supervision of convalescent typhoids, who may be passing with every micturition pure cultures of the typhoid bacillus?

These patients were given careful instruction as to the disinfection of their urine and were urged to send weekly specimens for further examination; but, as so often happens, they have carried out these instructions very unsatisfactorily. In 2 cases the urine 10 days subsequent to discharge still showed bacilli. In another case after a similar interval the organisms had disappeared. Two cases have yet to be heard from. It is, therefore, impossible to say just how long the bacilli may persist in these typhoid urines, but it is evident that they remain long enough to make the infected individuals a source of danger to those in their environment.

Neumann thought that the bacilli were finally removed mechanically. Assuming, as seems probable, that the large number of bacilli is due, not to excretion as such by the kidney, but to rapid multiplication in the bladder, it would seem that the post-febrile polyuria, with frequent and copious micturition, would, in the end, tend to eliminate the organisms entirely. This theory would explain, perhaps, the disappearance of the bacilli in Case VII. On the other hand Cases III and IX averaged during convalescence 70 and 80 ounces of urine daily, without, however, any apparent diminution in the number of bacilli. Nature does not, therefore, provide properly for this condition. Can we, by any method of treatment, render these cases innocuous?

My experience upon this point is confined to observations upon a single case (VI), in which the bladder was washed out with antiseptic solutions. At first boric acid was tried in the strength of 2 per cent, but, although the solution was retained for 2 or 3 hours, no effect upon the bacilli could be observed. Finally corrosive sublimate 1:7000 was injected, and although the fluid was passed out again almost immediately, no bacilli could be discovered during the 30 days following. It would seem, therefore, that in antiseptic irrigation of the bladder we have a possible means of freeing these cases of their dangerous character. Whether a like result can be obtained by the internal administration of salol or other similar drugs must be determined by further observation.

From a further examination of Table I it will be seen that 3 of the positive cases had been catheterized early in the disease. This offers the possibility, of course, of a typhoidal infection from without. The fact, however, that in these cases the bacilli were always found in pure culture and unaccompanied by colon bacilli or other bacteria would, as it seems to me, speak almost certainly for their internal origin.

Case IX is especially interesting as having developed during convalescence an epididymitis. A venereal origin, gonorrhœal or otherwise, was entirely improbable from the history, and the most plausible theory was that the affection of the epididymis was typhoidal in character, having its origin in the infected urine. The inflammation subsided without surgical interference, so that conclusive bacteriological investigation was impossible.

To proceed with the analysis of the tables, we find that the presence of bacilli in the urine was practically always accompanied by albuminuria, but that in nearly all the cases the amount of albumin was small, in only one case reaching ⅛ per cent.

Of the 29 cases in which the urine was negative, so far as typhoid bacilli were concerned, 15 had albumin in the urine, and that, too, in amounts larger, on the average, than those containing the typhoid organisms (XXXII, ¼ per cent; XXXV, ⅙ per cent).

As to the presence or absence of casts, they were found in all albuminous urines except those of Cases I and VIII. The record of Case I is decidedly incomplete, however, and it is more than probable that casts were present at some time during the disease.

No parallel can be drawn, therefore, between the presence of albumin and casts and the presence of typhoid bacilli in any urine. On the other hand we can say that a non-albuminous urine very probably contains no typhoid bacilli. Case III was the only exception in this series, showing no albumin upon the 41st day in the presence of many typhoid organisms. Furthermore, Case VII demonstrates the fact that albumin can persist after the disappearance of the bacilli.

Of the 29 cases in which no typhoid bacilli were found, 8 showed bacteria of other kinds. Of these 8 cases, 4 (XVII, XIX, XXVIII, XXXV) may be assumed to have derived their organisms from within the body. In the other 4 cases (XIII, XXII, XXVI, XXXII) the bacteria may have been of external origin (catheterization, operation). In these 8 cases a diplococcus (not further identified) was found in 2 (XIX, XXVIII); colon bacilli alone in 4 (XXII, XXVI, XXXII, XXXV); a diplococcus followed by colon bacillus in one (XIII); a streptococcus followed by colon bacillus in one (XVII). The colon bacilli presented no special points of interest except that isolated from Case XXVI. This organism was moderately motile, and grew upon litmus-milk, potato, gelatin and sugar-agar in a manner exactly similar to that of the typhoid bacillus. It might, therefore, according to criteria in vogue previous to 1890, have been called the typhoid organism. It was found, however, that upon peptone solution the bacillus produced immense amounts of indol; that it failed to react to typhoid serum; that it grew with acid reaction upon solution No. 1 of Proskauer and Capaldi, and that it failed to produce general cloudiness upon the tube medium of Hiss. This organism illustrates, then, forcibly the point referred to above, namely, that the results of investigations made in the eighties must be taken with a certain amount of reserve.

Cystitis was present in 7 cases and, with one exception (V), was always associated with bacteria and with a history of catheterization or operation. On the other hand the urine of 11 cases contained many bacteria with no evidence of cystitis.

In a general way the cases which showed typhoid bacilli in the urine were of a more severe type, as evidenced by the clinical course and mortality. Especially to be noted is the tendency to relapse and complication (I and IX).

The finding of typhoid bacilli in the sputum (I) is of interest in its possible etiological relation to the pneumonia which was present in this case.

Finally, the presence of typhoid organisms in the urine casts doubt, from a strictly scientific point of view, upon the work which has been done upon typhoid stools. Certainly, in my own investigations of the fæces in typhoid fever,* I never took into consideration the possible origin of the bacilli from the urine, and no effort was made to exclude the urine from the specimens examined. Nor have other observers, so far as I am aware, mentioned this possibility of error. From the point of view of diagnosis, of course, it makes no difference whether the bacilli come from the fæces or the urine.

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* *Boston Med. and Surg. Journal*, Oct. 28, 1897.

CONCLUSIONS.

(1) Typhoid bacilli were demonstrated in the urines of 9 out of 38 typhoid patients (about 25 per cent). 172 specimens were examined with 44 positive results.

(2) The bacilli, when demonstrated, were always present in large numbers and in practically pure culture.

(3) The bacilli appeared first in the later stages of the disease and persisted in the great majority of cases far into convalescence.

The urines of typhoid patients should, therefore, not only be rigidly disinfected during the disease, but they should also be carefully supervised during convalescence.

(4) The typhoid bacilli were practically always associated with albuminuria and the presence of renal casts. On the other hand urines containing considerable amounts of albumin and casts in large numbers often showed no typhoid bacilli.

(5) Irrigation of the bladder with antiseptic solutions offers a possible means for removing permanently the bacilli from the urine.

In conclusion I wish to express my indebtedness to Dr. J. H. Wright, Director of the Pathological Laboratory, and to the physicians of the visiting and house staff at the Massachusetts General Hospital, for their kind co-operation in the work.

cases out of 7), although, in this instance, the more important of the modern differential tests were used.

The following details of my own investigations and results will be found to confirm substantially the conclusions stated above.

The methods employed in the work were briefly these: In the case of male patients (by far the larger number) the glans penis was first thoroughly cleansed with corrosive sublimate solution 1:1000. The urine was then passed in two portions into sterilized test tubes. Only the second portion was used for bacteriological purposes. The female patients were catheterized with all possible antiseptic precautions, and in these cases also only the second part of the urine was used.

The urine obtained in this manner was then plated, with as little delay as possible, upon plain agar. Two plates were made for each specimen, one representing a single loop of urine and the other 1 cc. (approximately). After being left for 18 to 24 hours in the incubator the plates were examined under a low power of the microscope (Zeiss AA or Leitz No. 3).

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The following tables give the results in detail. The numbers refer to the day of the disease as nearly as it could be calculated from the history.‡

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† In carrying out this test I have always made use of the dried serum, or "serum paper," described by me in the *Centralbl. für Bakt.*, xxi (1897), 445. The paper is now 14 months old and shows no appreciable decrease in its agglutinating power. It is certainly a most convenient form for preserving the test for laboratory use, especially in localities where typhoid cases are scarce, and where, therefore, fluid serum is not always to be had.

‡ I am indebted to the records of the Massachusetts General Hospital for my information as to the presence or absence of casts, and as to the clinical features of the cases.

regular arrangement of the rods and threads in cultures whereby marked resemblance to actinomyces colonies is produced, a resemblance still further increased by the presence of bulbous swellings on the free projecting ends of the tubercle bacilli. He concludes that the tubercle bacillus is to be placed in the same class of organisms as the ray-fungus and cannot properly be regarded as belonging to the schizomycetes. Since the publications of Fischel and of Coppen Jones a number of confirmatory observations, although with much difference of opinion as to their interpretation, have been published, among which may be especially mentioned the investigations of Bruns (8), Babes (9), McWeeney (10), Semmer (11), Marpmann (12), Johan-Olsen (17), Babes and Levaditi (13), and Friedrich (14). Babes and Levaditi, and Friedrich have succeeded in demonstrating in tubercles produced by intracranial and intravascular injection of tubercle bacilli typical ray-like colonies of the bacilli very closely resembling those of actinomyces.

Branching, filamentous and bulbous forms of tubercle bacilli have been observed most commonly in old cultures, although they have been seen by Fischel, Coppen Jones and others in material from tuberculous cavities and in sputum. Recent observations, as already mentioned, show that under certain special conditions they may occur also in the tissues. In view of the interest of the subject and the relatively small number of reports of finding branched forms in the sputum, it has seemed to me desirable to record my observation of such forms in this situation.

The sputum was from a young woman suffering from rapidly advancing pulmonary phthisis. The expectoration was abundant and of offensive odor, containing, besides numerous bacilli, large quantities of elastic tissue.

The stained specimens were prepared as follows: A minute portion of the sputum was spread upon a cover-glass and another glass drawn gently over the first; the two specimens thus obtained were allowed to dry, passed rapidly three times through the alcohol flame, and then stained several hours, generally twenty-four, with the following solution:

Fuchsin	1 part.
Alcohol	10 parts.

Dissolve the fuchsin in the alcohol and add 100 parts of a 5 per cent aqueous solution of carbolic acid.

When rapid staining was desired a portion of the staining solution was poured into a watch-glass, the specimens to be stained being placed face downward upon it; the watch-glass was then gently heated until steam was given off freely, then placed aside and the specimens allowed to stain for about ten minutes longer. After staining, the preparations were washed in distilled water and decolorized with a 20 per cent nitric acid solution in alcohol; they were then washed in alcohol, dried and mounted in balsam or examined directly. Specimens stained by either of these methods gave excellent results, the tubercle bacilli being colored a very dark red, all else being decolorized.

In almost every preparation made from this sputum there were observed several bacilli showing true branching, and also those showing what I believe to be intermediate stages between the simple and the branched rod. While the typical straight or slightly curved bacilli are, of course, much the more numerous, the branched and intermediate forms are present in almost every microscopic field and are easily demonstrated. The appearances presented by the various branched and intermediate forms are very numerous and it would be impossible to depict them all, but the drawings reproduced in Plate XXVIII show the more striking variations.

In very many of the preparations there may be seen bacilli like those shown in Plate XXVIII, Fig. 1, and which are evidently false branching forms. On casual examination the resemblance which these bacilli (for they are multiple) bear to the true branching form is very striking, and it needs very careful focussing and good illumination, in many instances, to distinguish the difference. This appearance is due not to a single bacillus sending off branches, but to two or more bacilli lying very closely together and in such a position as to give the impression of true branching. Careful examination will, however, in almost every instance disclose a slight, unstained interval at the point where the suspected branching takes place. Such a minute examination should be made of every bacillus which appears to branch. Where no unstained interval can be detected by reason of one end of the bacillus overlapping another, careful focussing will show a difference in the level of the bacilli where they overlap, and also a deeper staining at that point, whereas in the true branching form no such difference in level or staining can be detected. Another distinction exists in the fact that in the false-branching type the bacilli forming the supposed branches are of the same length as the parent germ, whereas in the branching form the branches may be much longer or shorter than are the bacilli from which they spring.

Of the true branching bacilli several forms may be observed, which are doubtless simply stages in the growth of the organism. In certain fields there are seen bacilli which show at one or more points small knob-like projections, which cause a marked bulging in the outline of the organism. This form is illustrated in Plate XXVIII, Fig. 2, and evidently indicates the very first stage of branching, *i. e.* budding. These budding forms were not numerous in the preparations examined, but occurred in small numbers in the majority of the microscopic fields. They need sharp focussing and a good light to be distinguished, as in some of them the bulging is very slight and only such careful examination will demonstrate them. The bulging or knob is generally situated laterally, but may be central. The bud, as it may be called, stains like the rest of the bacillus, and sometimes a very small, oval, unstained speck may be seen within it which resembles the unstained portions in the bacillus. Generally, however, no such appearance is present, the bud staining uniformly throughout. Is this unstained oval speck a spore? I believe not, any more than are the similar unstained spots which occur in tubercle bacilli in general and which are not regarded by the best observers as spores.

The budding form just described is of interest, as it is doubtless the first stage of branching, and I believe it is present more often than is generally known. I have examined a large number of specimens of phthisical sputa since I first observed this form and have found it present in about 15 per cent of the cases, although in none was the branched form present, save in the one case described in this paper. This result may appear contradictory if we assume that the budding is the first stage of the branching form, but it is probable that some unknown factor has hindered the further development of the process of branching.

The next stage in the development of branching is shown in Plate XXVIII, Fig. 3. All the preparations made from the sputa in this case show numerous examples of this form. Here the little knob-like outgrowths, or buds, have developed into short branches, which vary in number in different bacilli, some giving off only one branch, while others give off two or more. The branches, at this early stage, are generally shorter than the parent bacillus and stain, almost without exception, uniformly throughout; the parent bacillus, however, generally shows intervals which take the stain very faintly or not at all. The bacillus itself, regardless of the branches, is much longer than usual, and somewhat more curved; they stain more deeply than the normal bacilli and resist a stronger decolorizing solution.

In nearly all the preparations of this sputum, the next stage, the fully developed branching form, can be seen (Plate XXVIII, Fig. 4). We have here simply a more advanced stage of the process, the short branches having developed into those which are as long as, or longer than, the parent bacillus. Of these fully developed branched forms there are present many varying examples; sometimes only one long branch is given off, sometimes two or more, sometimes one or more long branches and several minute buds; occasionally bacilli are seen where the primary branch has given off one or more secondary branches.

At *a* in Fig. 4 may be seen a bacillus which presents a well developed branch and three bud formations; this clearly indicates that the "bud" is the preliminary stage of the branch. It will be noticed that whereas the "buds" have taken the stain very deeply, the fully developed branch is stained much more faintly.

At *b*, in the same figure, is illustrated a bacillus presenting two short branches and one bud formation. Some of the bacilli are very fantastic in appearance, being much curved and giving off one or more branches, also curved (*d*, Fig. 4). Nearly all the bacilli present well marked stained and unstained areas, but it is noticeable that the branches present fewer of these, although some show unstained areas, irregular in outline. The bacilli are much longer than usual and many of the preparations show single filaments which are very long. In one preparation a peculiar appearance is noted (*g*, Fig. 4), which seems to represent the union of two branched bacilli by an intervening branch. This probably is an exaggeration of sub-branching. Many of the bacilli show sub-branching, the original branch giving off one or more smaller branches.

It is possible, then, to trace in these preparations from sputa the process of branching as it occurs in the bacillus tuberculosis. The bacillus destined to present this change becomes longer than usual and presents one or more knob-like outgrowths somewhere along its course; these outgrowths gradually lengthen and at last become true branches, which may, in turn, give off other branches.

We are not informed as to the special conditions which determine the development of branching forms of the tubercle bacillus. They have been most frequently observed under unfavorable conditions of environment, as in old cultures. In the present case it may be that similar factors are involved, as the rapid breaking down of tissue which characterized the process in the lungs may have exhausted the pabulum for the numerous bacilli present. Still the recent observations of Babes and Levaditi and of Friedrich show that similar forms may occur in fresh miliary tubercles. Various theories have been advanced as to the significance of the branching, filamentous and club-shaped forms of the tubercle bacillus.

Some have considered these to be simply degenerative or pathological forms of the bacillus and without significance as to the classification of the organism. This is the view advocated by A. Fischer (15) in his "Vorlesungen über Bakterien," who places these forms of the tubercle bacillus, as well as similar ones of the diphtheria bacillus, in the group of involution forms. There are several objections to this view, of which it will suffice to mention that the forms in question are not limited in their occurrence to old cultures and unfavorable conditions of environment.

The opinion more generally advocated is that the occurrence of these forms necessitates some change of formerly accepted views as to the classification of the micro-organisms concerned. Thus Klein suggests that the branching is a reversion to an ancestral type which presented mycelial growth.

Fischel pointed out the analogies between actinomyces and the tubercle bacillus, and Coppen Jones holds that both belong to the same group of organisms, but he leaves undecided whether this is among the hyphomycetes or some other class. He suggests that there may be some as yet unknown saprophytic mode of existence of this bacillus. He suggests the name Tuberculomyces (hominis, avium, etc.) for the bacillus.

Bruns regards the branching shapes as involution forms when observed under parasitic conditions. He says "they pertain to the saprophytic growth-form of a higher organism which, as a parasite in the animal body, appears in the form of rods." Marpmann, accepting the interpretation of Bruns, employs the designation Mycobacterium tuberculosis, a name adopted by Lehmann and Neumann in their "Grundriss der Bakteriologie."

The sharper differentiation within recent years of the streptothriceæ, cladothriceæ and allied organisms has led several writers to express the opinion that the tubercle bacillus and the diphtheria bacillus are very closely allied to the streptothriceæ, and some hold that they should be transferred to this group. Kruse (16), in the last edition of Flügge's "Mikroorganismen," discusses this question and, although he does not venture to place the bacteria in question under the streptothriceæ, he considers them to be very closely related to actinomyces and other organisms belonging to this group. Johan-Olsen (17) places the tubercle bacillus under the streptothriceæ and accepts the name Tuberculomyces. The streptothrix group is characterized especially by branching, filamentous forms of organisms, and is placed between the bacteria proper and the hyphomycetes, being more closely akin to the former than to the latter.

It is evident that further investigations are needed in order to enable us to come to any definite conclusion as to the significance of the branched forms considered in this paper, and for the present it would seem to be premature to adopt any radical change in the accepted classification of the tubercle bacillus and the diphtheria bacillus.

DESCRIPTION OF PLATE XXVIII.

Fig. 1. False branching form of the Bacillus tuberculosis in sputum. Careful examination shows an appreciable interval between the organisms. × 1000.

Fig. 2. Budding form of the B. tuberculosis in sputum. The little elevations or " buds " are probably the earliest evidence of branching. × 1200.

Fig. 3. Branching forms seen in sputum. × 1200.

Fig. 4. Various branching forms in sputum. × 2000.

a. An organism presenting one branch and three bud formations.

b. Organism showing two branches and one bud formation.

c. Organism showing the stained and unstained areas in its protoplasm.

d. Organism presenting two branchings, one of which stained uniformly, the other presenting unstained areas.

e. A branched form of the bacillus.

f. A bacillus showing one branch and two sub-branches, with another branchless bacillus lying beside it.

g. A peculiar formation probably due to the junction of two branched bacilli.

h. Y-shaped form of the organism, showing two bud formations.

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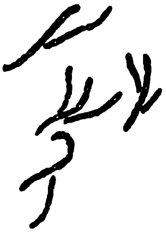


FIG. 1.

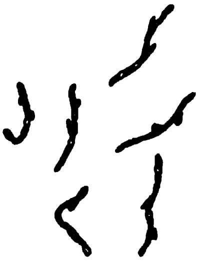


FIG. 2.

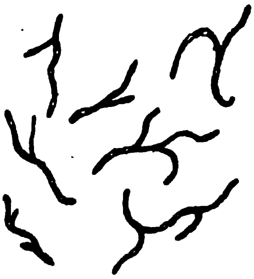
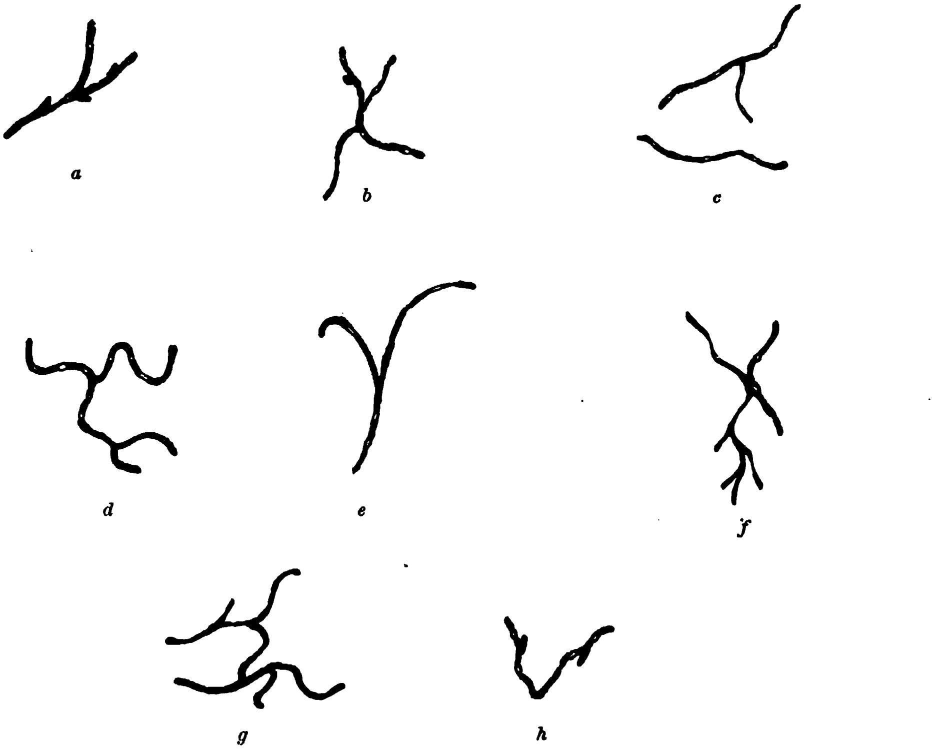


FIG. 3.



a b c d e f g h

FIG. 4.

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THE EFFECTS UPON THE BLOOD OF THE TUFNELL METHOD AND THE CALCIUM SALTS IN THE TREATMENT OF AORTIC ANEURISM.

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The following studies constitute a partial report of investigations which have been undertaken with the hope of determining the exact effects upon the blood of the current treatments of aortic aneurism. Drs. Pepper and Tyson placed at my disposal three such cases, one of whom died of rupture before the studies were completed, so that the report comprises but two cases.

The capillary and venous blood of the patients was first studied before any treatment was instituted, the natural volume of their urine noted, and the renal elimination of calcium accurately determined. The patients were then placed under treatment and the same determinations again carried out. In each case two sets of determinations were carried through both before and during treatment, with the exception of the estimations of urinary calcium and of the coagulation time of the capillary blood, which were performed every three days while the investigation was in progress.

The treatment consisted of rest, a dry diet, restriction of liquids, regular venesections (every 18 days), and the internal administration of potassium iodide grm. i and calcium chloride grm. ii daily. The use of a dry scant diet, restriction of liquids, and venesections are old therapeutic ideas, employed especially by Valsalva and Albertini; it is, however, due to the advocacy of Tufnell that they have held sway in modern practice. These measures have been held to reduce the volume of the circulating blood and to increase its coagulability. It cannot be believed today that therapeutic venesection more than momentarily diminishes the volume of the circulating blood, so quickly is the amount abstracted replaced by fluids from the tissues; in fact it is not improbable that the lymph current may more than replace the volume withdrawn by the bleeding. For the rest there is more definite evidence. Withdrawal of all drink or profound catharsis soon leads to an oligohydræmia. I have, however, been able to find no studies tending to show that the degree of limitation of fluids practised in cases of aneurism has actually caused such an inspissation of the blood. Tufnell's own regimen did not include over 375 cc. of liquid in the food and drink, which under ordinary circumstances would not furnish over 300 cc. of urine. Such a regimen is extremely distressing. In the cases here reported it was not possible to reduce the patient's liquids below the urinary equivalent of 400 to 500 cc. per diem, and even that was very irksome. Whether or not a reduction of the water in the circulating blood renders it more coagulable is entirely an open question; I know of no experimental work tending to show that simple oligohydræmia *per se* is accompanied by an increase in the coagulable properties of the blood. Potassium iodide was administered for the relief of pain, without expectations of any action upon the blood. The employment of calcium salts to heighten the coagulating properties of the blood, so warmly advocated by Wright, is based upon the now generally admitted fact that calcium bears in some way an integral relation to the molecule of fibrin.

The methods employed by me were as follows: The calcium in the urine was estimated by weighing as calcium oxide after precipitation as calcium oxalate, according to established procedures. The specific gravity of the blood was determined by accurate weighing in a picnometer of a capacity of about 12 grm. of water. The calcium in the blood was estimated by evaporating about 75 cc. of blood to dryness; the residue was then incinerated, the ash dissolved in hydrochloric acid, diluted and neutralized; the phosphates were then removed by the addition under heat of ferric chloride in the presence of acetic acid and sodium acetate; the calcium in the filtrate was then precipitated as the oxalate, reduced to the oxide by heat and weighed.

The volume of plasma and corpuscles was determined by the Bleibtreu method. In this method two or more portions of blood are mixed in different known proportions with an isotonic * saline solution, the cells allowed to sedimentate, and the nitrogen of the clear plasma in the different mixtures estimated by the Kjeldahl method or the specific gravity determined. Since the respective quantities of blood and saline solution are known, and since the dilution occurs (theoretically) in the plasma alone, the volume of the plasma, and from it that of the corpuscles, is calculated according to the following formula:

$$N^2\frac{s^2}{b^2}-N^1\frac{s^1}{b^1}=(N^1-N^2)x.$$

N^1 = nitrogen of mixt. 1. N^2 = nitrogen of mixt. 2. b^1 and b^2 = volumes of blood in the dilutions. s^1 and s^2 = volumes of solution in the dilutions. Technically this procedure is accurate. In principle, however, it is not accurate unless the diluting fluid have the same osmotic density as the plasma of the particular blood under consideration, otherwise there will be an exchange between the cells and the plasma.† Whether this exchange is always in one direction or not has not been determined; so far as clearly demonstrated, the exchange seems to be from the cells into the plasma. The extent of this exchange is certainly not marked; the amount of nitrogen involved is so small in comparison to the quantity of nitrogen in the native plasma that the method with its acknowledged error remains much more accurate than those of sedimentation and centrifugation. The error was minimized, I believe, by diluting the blood with small quantities of saline solution to which potassium oxalate was added to prevent coagulation, and then quickly removing the cells by centrifugation so that only a few minutes elapsed from the time the blood was drawn from the vein until the plasma was transferred to the Kjeldahl digestion flasks. The diluting fluid employed was a 4 per cent oxalate solution in a 0.9 per cent NaCl solution. The first mixture was of large quantity, since it also served for the estimation of the fibrin, usually 4 cc. of the solution to about 96 cc. of blood; the second mixture was usually in the proportion of 2 parts of the solution to 25 parts of blood. I have often

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* The word "isotonic" is here employed in its exact sense, related to osmotic density.

† If the osmotic density of the plasma of the particular blood be determined, preferably by the estimation of the reduction of the freezing point, and the saline diluting fluid made to correspond thereto, the result will be accurate. In the use of such solutions whose osmotic density corresponds to that of human plasma on an average (as 0.91 per cent. sodium chloride or 1.42 per cent. sodium sulphate) there will in the individual cases probably always be some exchange between the cells and the diluted plasma.

made smeared preparations of the corpuscles at the moment when the diluted plasma was drawn off for nitrogen analysis and found the corpuscles as a rule of normal appearance and staining reactions. At the end of an hour, however, the appearances of the red cells began to be altered, and these alterations progressed as time elapsed, and corresponding thereto later analyses of the plasma have always given higher quantities of nitrogen. I likewise made comparative determinations with the hæmatokrit, both with diluted and undiluted blood, and the results convinced me that the Bleibtreu method amply repays in accuracy the labor it involves.

From the plasma-nitrogen the amount and percentage of proteids in the plasma may be calculated, and in connection with the volume of plasma the amount of albumin in the plasma of 100 cc. of blood may be easily determined. These factors are of value, since any alteration in the quantity of water in the plasma would at once affect the quantity of albumin. There is in normal plasma a small quantity of non-proteid nitrogenous substances. In my cases the plasma has on an average contained azotized substances not precipitable by tannic acid corresponding to 0.060 N in 100 cc. of plasma, and that amount has been regularly deducted from the plasma-nitrogen before converting it into terms of albumin.

The fibrin was estimated by the recent method of Kossler and Pfeiffer.* A known quantity of venous blood, about 100 cc., is rendered noncoagulable by the addition of from 4 to 6 cc. of a 4 per cent solution of potassium oxalate in 0.9 per cent NaCl solution. The cells are immediately removed by centrifugation and the plasma-nitrogen determined by the Kjeldahl method (this also serves as mixture 1 of the Bleibtreu method). To 20 cc. of the oxalate plasma are then added 3 cc. of a 2 per cent calcium chloride solution. Spontaneous coagulation occurs rapidly; following the complete separation of the fibrin and the serum, the nitrogen of the serum is determined by the Kjeldahl method. Since the volume of plasma, the oxalate dilution of the blood and the calcium dilution of the oxalate plasma are known, the difference between the plasma-nitrogen and the serum-nitrogen with consideration of the aforementioned factors represents the nitrogen of the fibrin in the native volume of blood, the formula being as follows:

$$Nf = \frac{v}{v - v^{1}}\left(Np - Ns\,\frac{p + c}{p}\right).$$

Nf = fibrin nitrogen in 100 cc. blood. v = volume of oxalate plasma

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* Kossler and Pfeiffer, *Centralbl. f. innere Med.*, xvii (1896), 1.

(Bleibtreu method). v^1 = amount of oxalate solution in 100 cc. of oxalate blood. Np = nitrogen in 100 cc. of oxalate plasma. Ns = nitrogen in 100 cc. of the diluted serum. p = quantity of oxalate plasma in the artificial coagulation. c = quantity of calcium solution in the same. Since we do not know the formula for the molecule of fibrin, the findings must be expressed in terms of nitrogen. This method is very accurate, though technically quite difficult.

The time of coagulation was determined by Wright's method. A dozen glass tubes of ¼ mm. dimensions are supported in glass or rubber cases and suspended in water kept at body temperature. An assistant noting the time, the numbered tubes are successively employed as follows: from a deep puncture in the finger the blood is drawn one-third the length of the tube, which is then removed from the finger and the column of blood drawn up so that it occupies the middle third of the tube, which is then replaced in its warm case. After a lapse of about two minutes (a preliminary orientation is always advisable) the tubes are successively blown out and the coagulation point noted, the time of which can then be calculated from the records. The range of error in this method is fully forty seconds, that is, it may be 20 seconds to either side of the given result. From studies on normal male adults I have found the time of coagulation with this method to range from 1.5 to 3 minutes, the average being a little lower than Wright's figures, 2.5 minutes. It may not be amiss to say that this seemingly simple method requires not a little practice if constant results are to be obtained.

The results of the observations are presented in the tables on pages 380-1.

X in case Brown indicates the beginning of the treatment, XX indicates the withdrawal of the calcium, XXX its resumption. X in case Griffith indicates beginning of treatment; XX indicates its rigorous enforcement, since the urine gave evidence of neglect of orders.

The average output of urine of Brown under voluntary conditions was always over 1100 cc.; under the Tufnell regimen it averaged 440 cc. Griffith's urine naturally fluctuated from 950 to 1300 cc.; under the treatment the average daily output was 525 cc. Prior to beginning treatment both patients were upon the same diet and drinking water, yet Brown eliminated by the urine only about 0.200 grm. $Ca_3(PO_4)_2$, while Griffith eliminated over 0.750 grm., a further illustration of the wide individual variations in urinary calcium elimination so often noted by different observers. In the case of Brown, despite the dry diet, the administration of calcium increased markedly the urinary elimination of that element, often to twice or thrice its natural quantity. Upon withdrawal of the drug (at XX) the quantity in the urine fell at once, and again rose promptly when its administration was resumed (at XXX). In the case of Griffith, the administration of calcium without strict enforcement of the dry diet (from X to XX) caused a considerable increase in its urinary elimination. But when the drinking was rigidly reduced the urinary elimination of calcium fell below the quantities previously eliminated while upon a normal diet without treatment. Even thus, however, the quantities eliminated were greater than in the case of Brown. It seems clear from these figures and from what is well known regarding the effect of the free use of water upon the absorption and urinary elimination of calcium salts, that were it held of prime importance to saturate the body with these salts, abundance and not restriction of water would be indicated.

That the ingestion of calcium affects the quantity in the circulating blood is suggested but not demonstrated by my analyses. Before the treatment, in the case of Brown, the quantities were 0.0172 and 0.0158 grm., during the treatment 0.022 and 0.0179 $Ca_3(PO_4)_2$ in 100 cc. blood; in the case of Griffith, before treatment, 0.018 and 0.0191 grm., during treatment 0.0146 and 0.015 grm., thus inclining to agree with the urinary elimination. It is clear, however, that so many other factors are concerned in this question that no conclusion can be established on this point.

The specific gravity of the blood in both cases was not materially affected by the treatment—1.0534, 1.051 before treatment, and 1.053, 1.052 during treatment, in the case of Brown; 1.060, 1.0607 before treatment, and 1.0598, 1.0609 during treatment, in that of Griffith. During the course of the treatment in both cases the number of red cells and the percentage of hæmoglobin diminished somewhat. Since the estimation of the specific gravity of the blood by weighing is much more accurate than the clinical methods of Thoma and Fleischl, I am compelled to look upon the apparent reductions either as erroneous or as due to peripheral conditions. Both the constancy of the specific gravity and the reductions in the results of the clinical blood examinations show that in these cases no diminution in the water of the blood was effected by the treatment.

The same result is clearly shown by the percentages of the volume of plasma in the volume of native blood. These were in the case of Brown, 61.75 and 64 per cent before treatment, and 66 and 64 per cent during treatment; in the case of Griffith, 57.4 and 56.8 per cent before treatment, and 57 and 57.2 per cent during treatment. Any oligohydræmia would result in a diminution of the volume-percentage of the plasma, and since in the case of Brown the variations were in the direction of hydræmia and in the case of Griffith the results were constant, it is again clear that in these cases no oligohydræmia was effected by the treatment.

The plasma-nitrogen, plasma-albumin (factor 6.38) and quantities of albumin in the plasma of 100 cc. of blood were quite constant in the case of Brown, except for the second estimation, in which all were noticeably low. The patient at that time was suffering from an attack of influenza, but since that was before the beginning of the treatment, and since the two later determinations under treatment agreed with the earlier one prior to treatment, the absence of changes is clearly shown. In the case of Griffith the results were constant, except in the third estimation, in which they were high. The causes of this are obscure, since the percentage-volume of the plasma and the specific gravity were not altered. That the nitrogen and albumin of the plasma should vary constantly with the volume of plasma and the specific gravity of the blood is, however, not to be expected, since so many other factors are concerned in the latter.

The fibrin-nitrogen was not increased by the treatment beyond the range of error of the method. The only notable variation was the reduction observed at the time of the second estimation of Brown (before treatment), 0.0593, when suffering from the attack of influenza already mentioned. It is, therefore, clear that the treatment in these two cases did not increase the quantity of fibrin in the native volume of blood. The gross phenomena of clotting were in all cases closely observed in the blood drawn apart for the estimation of calcium; in no case was the clot larger or firmer, nor did it retract or contract more quickly than normal.*

The time of coagulation as determined by Wright's method varied in the two cases. In Brown it was noticeably reduced during treatment below the figures noted previous to treatment, and reduced beyond the range of error of the method. During the six days when the calcium was withdrawn no differences in the time of clotting could be noted. In the case of Griffith, although there were marked variations both up and down, in general the time of clotting was not shortened. I believe, therefore, that in these two cases no acceleration in the process of clotting was effected by the use of calcium, since in Griffith, who best absorbed his calcium, no changes were noted, while in Brown the acceleration persisted while the drug was withheld. No inferences can be drawn respecting the influence of the Tufnell treatment upon the time of coagulation, since the results in the two cases do not agree.

The action of the calcium salts upon coagulation is and must remain purely empirical until the chemical relations of the process are better understood. Having apparently demolished the older views, Lilienfeld and his followers have in turn had grave doubts thrown upon their own by Pekelharing, Hammarsten and others,† and only thus much appears definitely known: that under certain circumstances, under the influence of several ill-understood factors, blood coagulates, and the product fibrin contains calcium and a proteid, plus how much more we know not, in a molecule of undetermined quantity. Under such circumstances discussion of the relations of calcium salts to blood coagulation under pathological circumstances would be profitless.

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* Realizing that terms of nitrogen do not convey to the general reader a clearly defined idea of quantity, I have converted the figures of nitrogen into terms of fibrin in order to make the results more tangible, using as a basis of calculation the formula of Schmiedeberg; this formula, however, can be accepted only in a provisional sense. The quantities of fibrin thus determined read as follows: Brown, before treatment, 0.464 and 0.355; during treatment, 0.433 and 0.475 grm.; Griffith, before treatment, 0.457 and 0.450; during treatment, 0.478 and 0.466 grm.

† For example, Hammarsten, *Zeitschr. f. phys. Chem.* xxii, 333, and Cramer, ibidem, xxiii, 74.

While it seems clear that in every point respecting the *modus operandi* of this mixed treatment for aneurism and its influence upon the blood the result was entirely negative, I wish most definitely to state that under the treatment both patients improved. Brown received but slight betterment, the dilatation seemed simply held in check. Griffith, however, improved markedly; the tumor and the physical signs and symptoms receded progressively until he was finally discharged with no symptoms and no signs except dulness beneath the manubrium and a faint bruit. Brown was kept constantly in bed. Griffith was allowed the liberty of the hospital and its grounds, so that in his improvement rest played no role.

TABLE I.—CASE, BROWN.

X indicates beginning of treatment; XX, withdrawal of calcium; XXX, its resumption.

	Quantity of urine in cc. per diem.	Urinary calcium $Ca_3(PO_4)_2$ in grm. per diem.	Blood calcium $Ca_3(PO_4)_2$ in grm. in 100 cc.	Sp. gr. of blood.	Percentage volume of plasma.	Plasma-nitrogen in 100 cc. plasma.	Plasma-albumin in 100 cc.	Albumin in plasma of 100 cc. blood.	Fibrin-nitrogen in 100 cc. blood.	Coagulation time in minutes and seconds.	Venesection.	Number of red blood corpuscles and percentage of hæmoglobin.*
	1040	0.1915	....	....	....	....	....	....	....	3.10	....	4820000, 85%
	1180	0.2164	0.0172	1.0534	61.75%	1.322 grm.	8.432 grm.	5.207 grm.	0.0776 grm.	2.50	300 cc.	4780000, 87
X	1070	0.2218	0.0158	1.051	64	1.165	7.433	4.757	0.0593	2.30	260	4620000, 83
	320	0.571	....	....	....	....	....	....	....	2.30	....	4750000, 80
	500	0.540	....	....	....	....	....	....	....	1.55	....	4620000, 80
	445	0.504	....	....	....	....	....	....	....	2.15	....	4780000, 85
	520	0.432	...	....	....	....	....	....	....	2.09	....	4720000, 80
	425	0.399	....	....	....	....	....	....	....	2.04	....	4760000, 82
	480	0.756	0.022	1.053	66	1.223	7.803	5.150	0.0725	2.18	250	4690000, 85
	425	0.416	....	....	....	....	....	....	....	2.25	....	4610000, 80
XX	420	0.246	....	....	....	....	....	....	....	2.01	....	4480000, 80
	380	0.185	....	....	....	....	....	....	....	2.09	....	4480000, 78
XXX	490	0.341	....	....	....	....	....	....	....	2.11	....	4580000, 75
	430	0.364	....	....	....	....	....	....	....	2.18	....	4520000, 78
	410	0.296	0.179	1.052	64	1.271	8.109	5.190	0.0795	2.05	270	4450000, 75

* Last two figures hæmoglobin %. Thoma-Zeiss and Fleischl apparatus.

TABLE II.—CASE, GRIFFITH.

X indicates beginning of treatment; XX, its rigorous enforcement.

	Quantity of urine in cc. per diem.	Urinary calcium $Ca_3(PO_4)_2$ in grm. per diem.	Blood calcium $Ca_3(PO_4)_2$ in grm. in 100 cc.	Sp. gr. of blood.	Percentage volume of plasma.	Plasma-nitrogen in 100 cc.	Plasma-albumin in 100 cc.	Albumin in plasma of 100 cc. blood.	Fibrin-nitrogen in 100 cc. blood.	Coagulation time in minutes and seconds.	Venesection.	Number of red blood corpuscles and percentage of hæmoglobin.*
	1160	0.860	....	....	....	....	....	....	....	2.15	....	5050000, 90%
	1240	0.889	0.018	1.060	57.4%	1.276 grm.	8.141 grm.	4.673 grm.	0.0765 grm.	2.20	260 cc.	4940000, 88
	1195	0.784	0.0191	1.0607	56.8	1.291	8.237	4.679	0.0752	1.55	250	4960000, 95
X	980	1.134	....	....	....	....	....	....	....	2.30	....	4910000, 88
	975	1.088	....	....	....	....	....	....	....	1.40	....	4930000, 85
	1210	0.972	....	....	....	....	....	....	....	2.02	....	4990000, 85
XX	580	0.652	....	....	....	....	....	....	....	1.59	....	4860000, 88
	550	0.720	....	....	....	....	....	....	....	2.10	....	4920000, 88
	560	0.380	0.0146	1.0598	57	1.351	8.619	4.913	0.080	2.08	225	4890000, 90
	525	0.535	....	....	....	....	....	....	....	2.08	....	4810000, 85
	400	0.498	....	....	....	....	....	....	....	2.12	....	4850000, 80
	625	0.671	....	....	....	....	....	....	....	2.50	....	4750000, 85
	490	0.574	....	....	....	....	....	....	....	1.59	....	4880000, 88
	460	0.560	0.015	1.0609	57.2	1.265	8.071	4.617	0.078	2.10	240	4830000, 85

* Last two figures hæmoglobin %. Thoma-Zeiss and Fleischl apparatus.

EXPERIMENTS ON THE APPLICATION OF THE ROENTGEN RAYS TO THE STUDY OF ANATOMY.*

BY ERNEST AMORY CODMAN, M. D., Boston.

PLATES XXIX-XXXVI.

The field of gross anatomy has been so well covered in the last century, and the dissecting knife and the method of frozen sections have enabled the anatomist to see so much of the internal structure of the body, that little has been left in this direction to the province of the Roentgen ray.

This does not apply, however, to pathological conditions of the bone in the living, for there the X-ray can do what the knife and frozen section cannot. For this reason it is important to have a knowledge of the normal X-ray anatomy of the bones, for the X-ray picture differs both from the photograph of the bare bones and from the frozen section, as it shows outline, internal structure and differences in density. The series † of skiagraphs which I have taken of the normal joints have their chief value from this point of view, as they show what may be considered the normal X-ray anatomy.

The skiagraphs accompanying this article were all taken from the cadaver (most of them from the same subject) by using a focus tube stimulated with a seven-inch spark induction coil. The current was about fifteen amperes, with a pressure of four volts in the primary coil. The break used was between copper surfaces immersed in distilled water.

In looking at skiagraphs there are several distortions and inaccuracies which must be borne in mind. One essential for a good skiagraph is an accurately focussed tube. The cathode rays are focussed by a concave aluminum electrode on the centre of a disc of platinum or the anode. The point where the stream of cathode rays strikes is the point from which the X-rays are generated. That this point should be as small as possible is important, in order that no penumbra should be formed. I have been fortunate in this series in having a tube in which this point was small, hence the outlines are sharply defined.

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* This paper is the report by the author to the Committee on the Bullard Fellowship of the Harvard Medical School, May 1, 1897.

† Referring to album presented with this report to the Committee on the Bullard Fellowship and now in the Warren Museum.

We may consider, then, that the X-rays proceed from practically a single point. In looking at a skiagraph we must consider it as a complete whole, made up of the shadows of the different parts of the object. The size and shape of the shadow of each part will depend on the relations of the distances between the point of light, the part of the object, and the plate. Thus the distance between the light and the plate remaining the same, the farther an object is from the plate the larger will be its shadow; and the farther an object, or part of an object, from the base of the perpendicular from the point of light to the plane of the plate the more will its shadow be distorted, the distortion being due to elongation in the direction of a line from the base of the perpendicular through the object.

When the object is at the same time away from the plate and away from the base of the perpendicular, both of these distortions will take place. Further, since each object or part of an object contains only a certain amount of shadow-casting material, the farther it is from the plate the greater will be the size and the less the density of the shadow.

An important corollary follows from this. A picture of a less dense object may be taken through a more dense, *e. g.* the ear through the skull. This can be done when the densities of the two objects are not greatly contrasted; but when one object is so dense as to be practically impenetrable to the rays, its shadow will of course obliterate the shadows of anything either in front of or behind it. This is generally true of metallic objects.

Another source of inaccuracy of these pictures is the fact that the rays in penetrating large objects are more or less diffused. This diffusion tends to blur the outlines of the parts of the object at a distance from the plate. It may also assist in making more distinct the objects in direct contact with the plate; for instance, in the picture of the ear taken through the skull, the diffused rays from the other parts of the head may help to take the picture of the ear, because they blur all those parts of the plate which are not protected by the ear, which comes in close contact with the wrapper.

It will be seen from the foregoing that in order to reduce these inaccuracies to a minimum the distance from the plate to the tube should be as great as possible, and that from the object to the plate as short as possible. The distance, therefore, must be gauged by the intensity of the rays and convenient time of exposure. We must also remember that the wider the object, or the deeper the object, the greater must be the distance between the plate and the tube. For instance, the skiagraph of a hand at ten inches is very little distorted, while that of two hands side by side would be considerably distorted, because the rays would strike the outer fingers at more acute angles.

A knee at ten inches, though little wider than a hand, would yet be considerably distorted, for it is so thick that the parts furthest from the plate would be much enlarged; the external condyle of the femur (the knee being on the inner side, for instance) would appear much larger than the internal (Fig. 1).

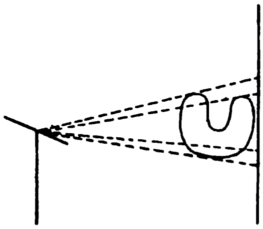

Fig. 1.

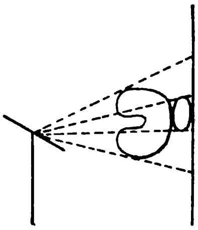

Fig. 2.

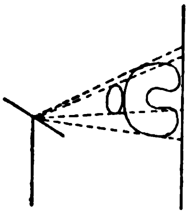

Fig. 3.

In order to reduce these distortions to a minimum the tube should be put as far away as is possible with a convenient time of exposure.

On the other hand, however, advantage can be taken of this distortion in taking a photograph of the patella. If the plate is put to the posterior side of the knee (Fig. 3) the shadow of the patella will be hardly, if at all, distinguishable. This would be true also if the plate were on the anterior side and the light far away, for the shadows of the femur would be dense enough to overshadow that of the patella. If, however, the plate be on the anterior side and the tube very near the posterior (Fig. 2) the shadow of the femur will be broadened and diminished in density, so that the shadow of the patella, which is but little magnified and as dense as before, will be seen through it.

The use of the fluoroscope is a practical way of determining the intensity of the rays. In determining the distance we must remember that the intensity of the light varies inversely as the square of the distance. In determining the time we can calculate that what we lose by doubling the distance we gain by multiplying the time of exposure by four.

THE STUDY OF THE MECHANISM OF THE JOINTS.

This has been undertaken in two ways. First, by skiagraphing the normal joints in their forced extreme positions, extreme flexion, adduction, etc. Secondly, by watching the movements with the fluoroscope. As the parts of the object near the plate show best, it has been necessary to take each position from both sides.

One thing which will at once arrest attention is the great distance that apparently intervenes between the bones. This is in part due to the fact that the articular cartilages, being easily traversed by the rays, do not cast a shadow. The accompanying skiagraphs of hands (Plates XXIX-XXXIV) were exposed at a distance of about twelve inches for 3 to 5 minutes.

The wrist joint has proved most interesting in this study, and the points brought out will be found in the following description:

For convenience we may consider the wrist joint to be made up of four immobile and two mobile elements.

I. Immobile (*i. e.* those made up of single bones or of a group of bones, the components of which cannot change relative positions). These are:

1. Metacarpal of thumb.
2. Metacarpal of ring finger.

3. Metacarpal of little finger.

4. Metacarpal of index and middle finger with trapezium, trapezoid, os magnum, and unciform.

This last group are so firmly attached to one another that they move as a whole, practically as one bone. No doubt, however, their ligamentous attachments allow of more or less spring in strained positions of the hand brought about by external force.

II. Mobile (the components of which change relative positions).

1. The intermediate row of carpal bones composed of scaphoid, semilunar, cuneiform and pisiform.

2. Radius and ulna.

From the skiagraphs it is found that the carpus and metacarpus are, in any of the extreme positions, in practically the same relation to the radius, no matter what the relation of the radius to the ulna, whether pronation or supination. This is due to the more or less flexible fibro-cartilage, which in any position completes the cup of the radial joint. The question of mechanism, then, is further simplified by leaving out the ulna, which really does not enter into the construction of the joint except as a pivot. The pisiform also does not enter the mechanism, serving only as a sesamoid for the ulnar tendons.

Proceeding to eliminate other accessory elements, we can disregard the metacarpals of the thumb, ring and index fingers, each of which moves independently on the large fixed element composed of the os magnum, etc. The thumb forms a typical saddle joint with the trapezium, with the pommels of the saddle so low that motion is allowed in a small circle, either as rotation within the circumference or straight motions on any of the radii. The metacarpal of the ring finger is allowed a slight antero-posterior motion of a few degrees. That of the little finger the same of slightly greater extent, with possibly a degree of adduction. This leaves us with:

I. The large compound fixed element of os magnum, etc.

II. The radius with fibro-cartilage.

III. The intermediate element of scaphoid, semilunar and cuneiform.

These constitute the real wrist joint, and their movements are very beautiful (Figs. 5 and 6).

The joint may be compared to a ball-bearing with the balls irregular and allowing only limited motions. It must be remembered, too, that there is no real separation of the component parts, for besides the ligaments tending to bind them together there is the atmospheric pressure which closes down the capsules and ligaments as each bone is drawn along another.

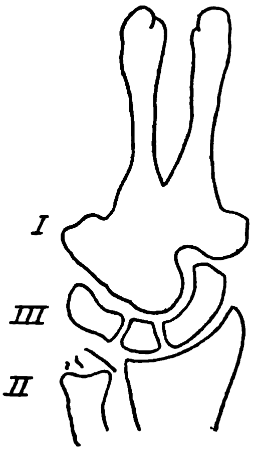

Fig. 4.

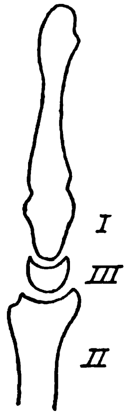

Fig. 5.

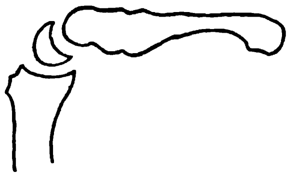

Fig. 6.

Starting from the straight position in antero-posterior view we put the joint in adduction (cf. Plates XXIX and XXX). The radius remaining fixed, the mobile intermediate row may move as a whole on the radius. This does happen to the extent seen in the skiagraphs. In the meanwhile the os magnum, etc., move on the intermediate row. This motion would be very slight in extent, since the outer side of the head of the os magnum comes against the lower end of the scaphoid, were it not for the fact that the mobile row is straightened out both by the pressure of the unciform on the cuneiform and of the magnum on the scaphoid (Plate XXX). The lower end of the scaphoid is also pulled down by the trapezium, so that it is extended. In fact, all the intermediate row in adduction are extended and in abduction flexed. This Dr. Dwight has beautifully shown by driving a long pin into each bone and watching the ends of the pins while manipulating the joint.

The os magnum element is also slightly flexed in adduction and extended in abduction to compensate for the opposite movements in the intermediate row.

In the same way the movements of flexion and extension are almost equally divided between the radio-carpal and intercarpal joint (Figs. 5 and 6 and Plates XXXII, XXXIII, XXXIV). The semilunar may be taken as the type of the intercarpal row and will be observed in the radiographs to tilt through an angle of about 90°. In like manner the os magnum tilts on it as it moves through an angle of 90°, giving to the whole joint its range of nearly 180° between the extreme positions.

This comparatively simple view of this beautiful mechanism is, I think, an accurate one, and the points are well confirmed by the anatomical specimens.

The chief value of the radiographs of the other joints is in forming a series for comparison with pathological conditions. The necessity for training in interpreting skiagraphs is evident from the foregoing description of the distortions of these pictures, so that an idea of the normal X-ray anatomy is necessary for an intelligent interpretation of abnormal or diseased conditions of the bones. The skiagraphs of the trochlear surface of the elbow joints form a good illustration of this, as it requires considerable study of the skiagraphs of the elbow taken from the side to enable one to make out the position of the bones.

The skiagraphs of the bones of the foot did not prove as interesting as I had expected, for the relative position of the bones of the tarsus remains almost the same in plantar as in dorsal flexion. Most of the motion takes place between the astragalus and tibia, while there is a slight general motion between the other bones which is pretty equally divided.

Many of these skiagraphs are doubly interesting, as they were taken from cadavers which had been injected with the usual mixture of starch and a red mercuric salt for coloring. The mercuric salt (red oxide) being more or less opaque to the rays, renders the arteries visible. The study of the arteries in this way has much the same advantages as those given by corrosions for the general grouping, regional distribution and relation to the bones (Plate XXXV). The smaller branches can be seen in a way impossible in dissection.

Other skiagraphs were also taken with metallic mercury injected into the joint capsules. Metallic mercury was found difficult to handle, expensive, and not capable of an even distribution in the cavities on account of its own cohesive tendency. A saturated solution of plumbic nitrate was therefore used instead and was found cheaper and more satisfactory.

I have spoken of the distortion of Roentgen-ray pictures, and particularly the fact that the parts of an object at a distance from the plate have their shadows magnified. Curiously enough this distortion can be very nearly corrected by the use of the stereoscope. Two skiagraphs may be taken of an object which, when placed in the stereoscope, will give one picture of that object which will have the appearance of solidity (Plate XXXVI). To take stereoscopic pictures two exposures are made with the plate and object in exactly the same relation to each other, but with the light in a slightly altered position in relation to both. This should be done by moving the tube to the right or left a distance corresponding to the normal space between the eyes or a little less, after taking the first skiagraph in the usual position. At the same time a new plate is substituted for the old one, care being taken to put it in exactly the same place.

In the stereoscopic pictures the skiagraphs were reduced to small "transparencies" and printed so as to make the bones and arteries appear white for greater effect. With a given pair of these stereoscopic pictures by transposing the prints in the stereoscope, *i. e.* placing them as (1-2) or (2-1), we can look at the object as if from behind or from before. For instance, in such skiagraphs as those of a hand we seem to look at either the palmar view or the dorsal, that is, the palmar arch will appear in front or behind the bone according as the one or the other of the prints occupies the right-hand space.

Now since the parts of the object farthest from the plate are enlarged, the best arrangement will be that which in the stereoscope will make us seem to look at the enlarged or near side of the object. The enlargement will then be that which would naturally exist on the retina in looking at the object from the side away from the plate. Thus the distortion is in part corrected. The criticism of these pictures, however, may justly be made that the parts of the object which show the clearest and with most detail are those which appear farthest off in the stereoscope. This of course is contrary to the simple optical effect. It is, however, not noticeable except in stereoscopic pictures of deep objects.

DESCRIPTION OF PLATES XXIX–XXXVI.

PLATE XXIX.

Wrist in straight position. Notice relation of scaphoid and semilunar to radius and compare Plates XXX and XXXI.

PLATE XXX.

Wrist in adduction. Notice leverage of head of os magnum on scaphoid and fulcrum between cuneiform and unciform. By this leverage the curve of the intermediate row is straightened out and becomes the arc of a larger circle. Cf. relations in Plate XXXI.

PLATE XXXI.

Wrist in abduction. Scaphoid is flexed and hence seems shorter than in Plate XXX, os magnum approaches scaphoid and the intermediate row becomes arc of a smaller circle.

PLATES XXXII, XXXIII AND XXXIV.

Wrist in straight position (Plate XXXII), extension (Plate XXXIII), and flexion (Plate XXXIV), as seen from side. The os magnum and semilunar show more clearly than the other bones, and may be taken as types of the two rows. By comparison of the three figures the division of the motion between the radio-carpal and intercarpal joint will be observed.

PLATE XXXV.

Adult male pelvis with arterial injection. There is a sound in the bladder and a scalpel under the left side of the cadaver. Reduced from 18 x 24 in.

PLATE XXXVI.

Stereoscopic views of hand with arterial injection. To obtain the solid effect one must look at the pictures cross-eyed, or with a stereoscope, having cut them out and transposed them.

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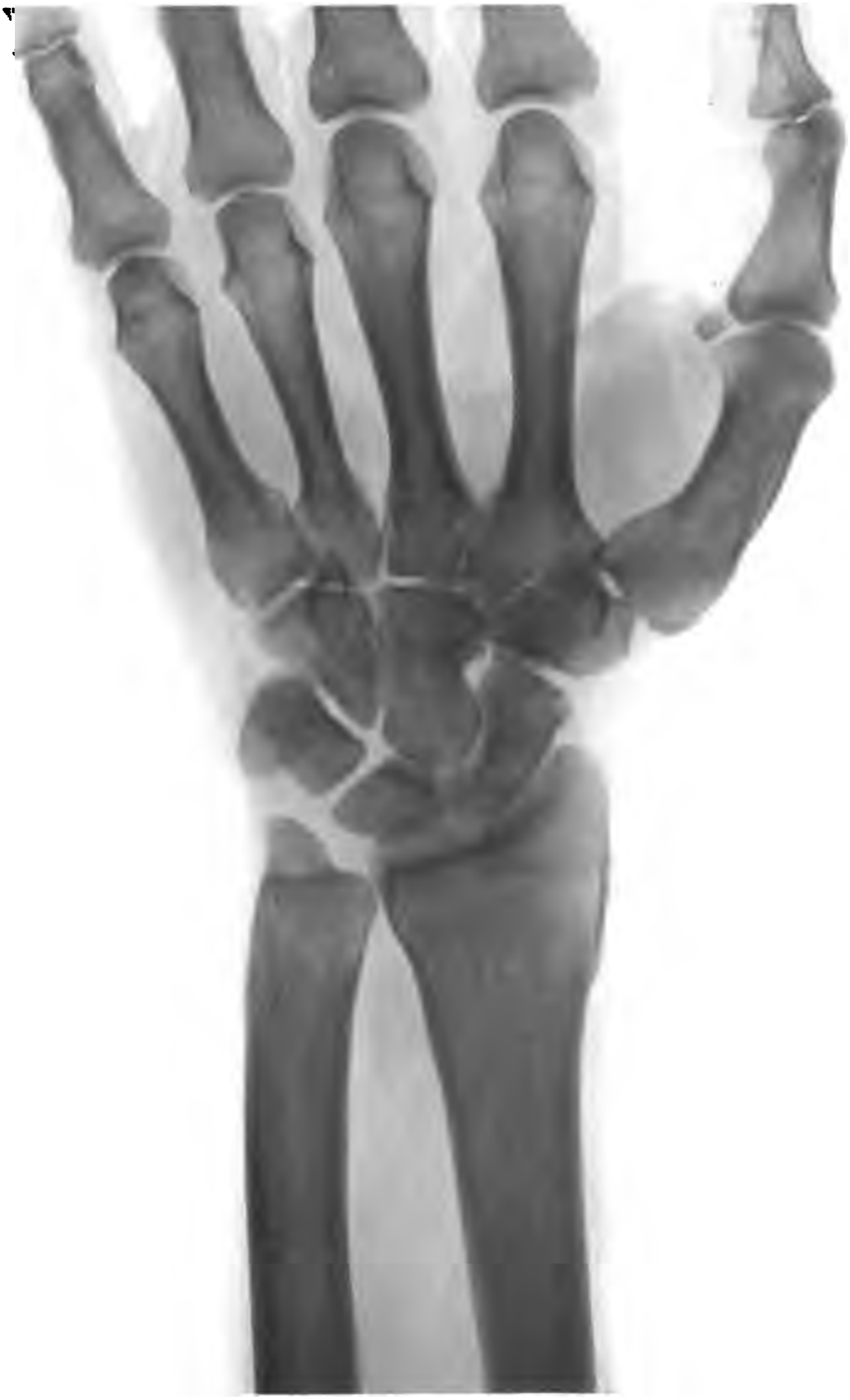

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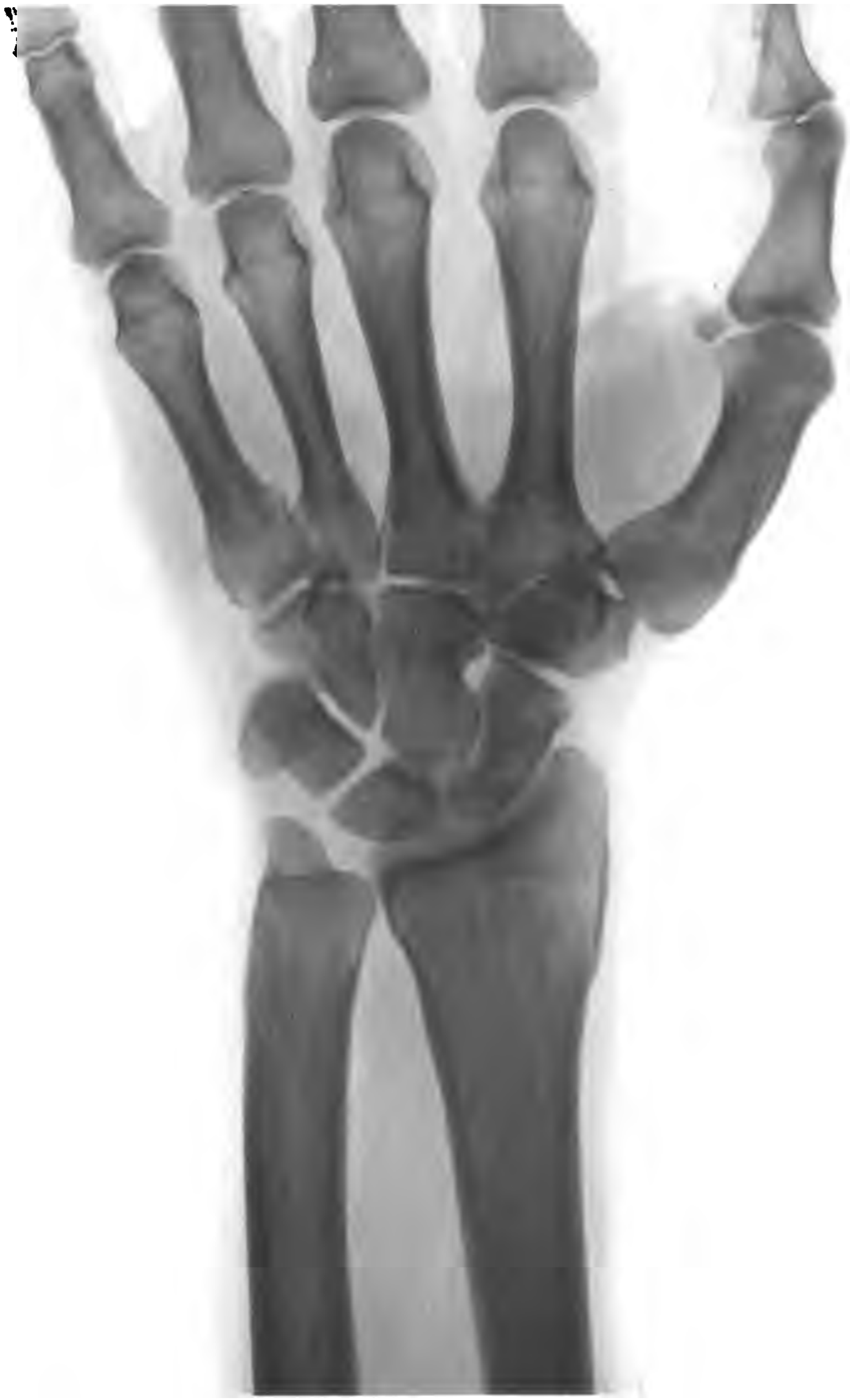

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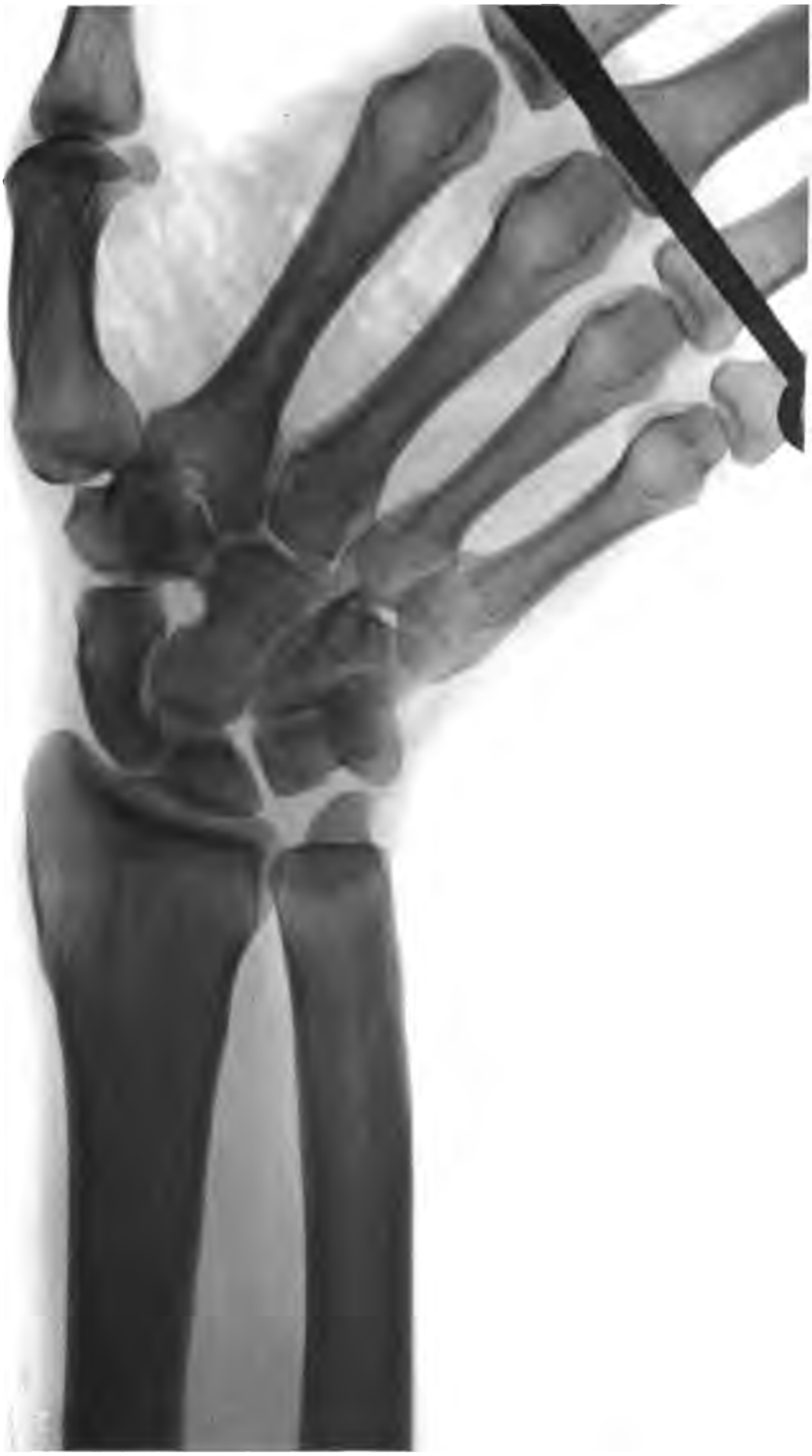
PLATE XXX.



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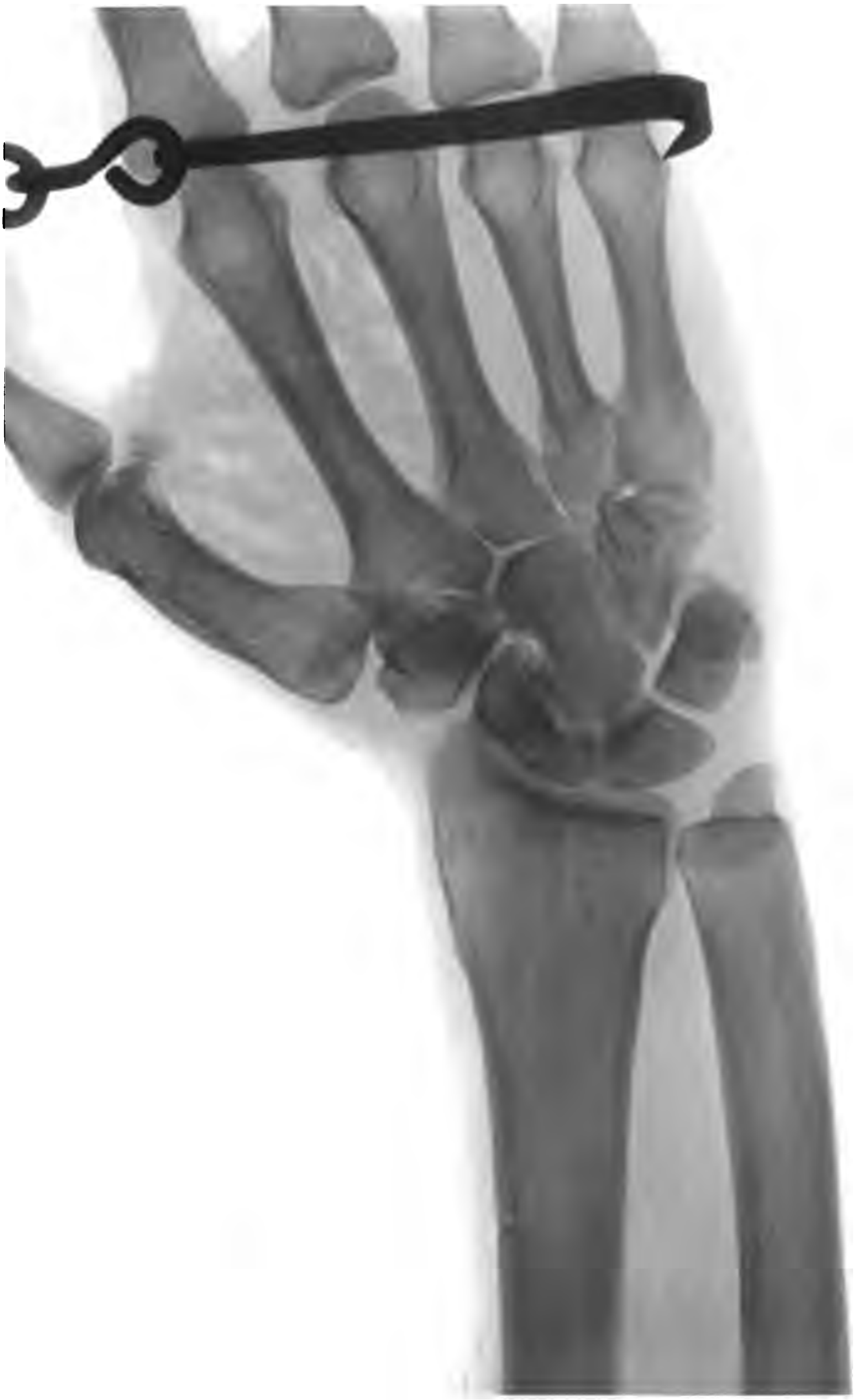

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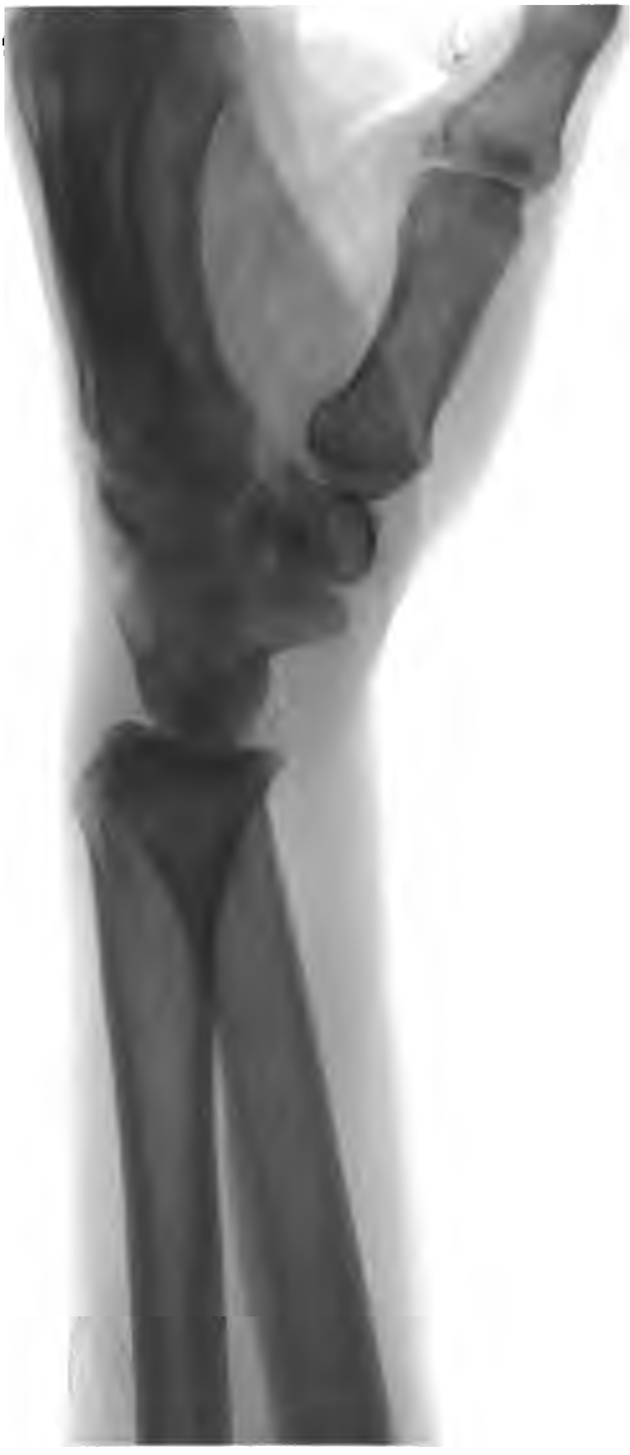

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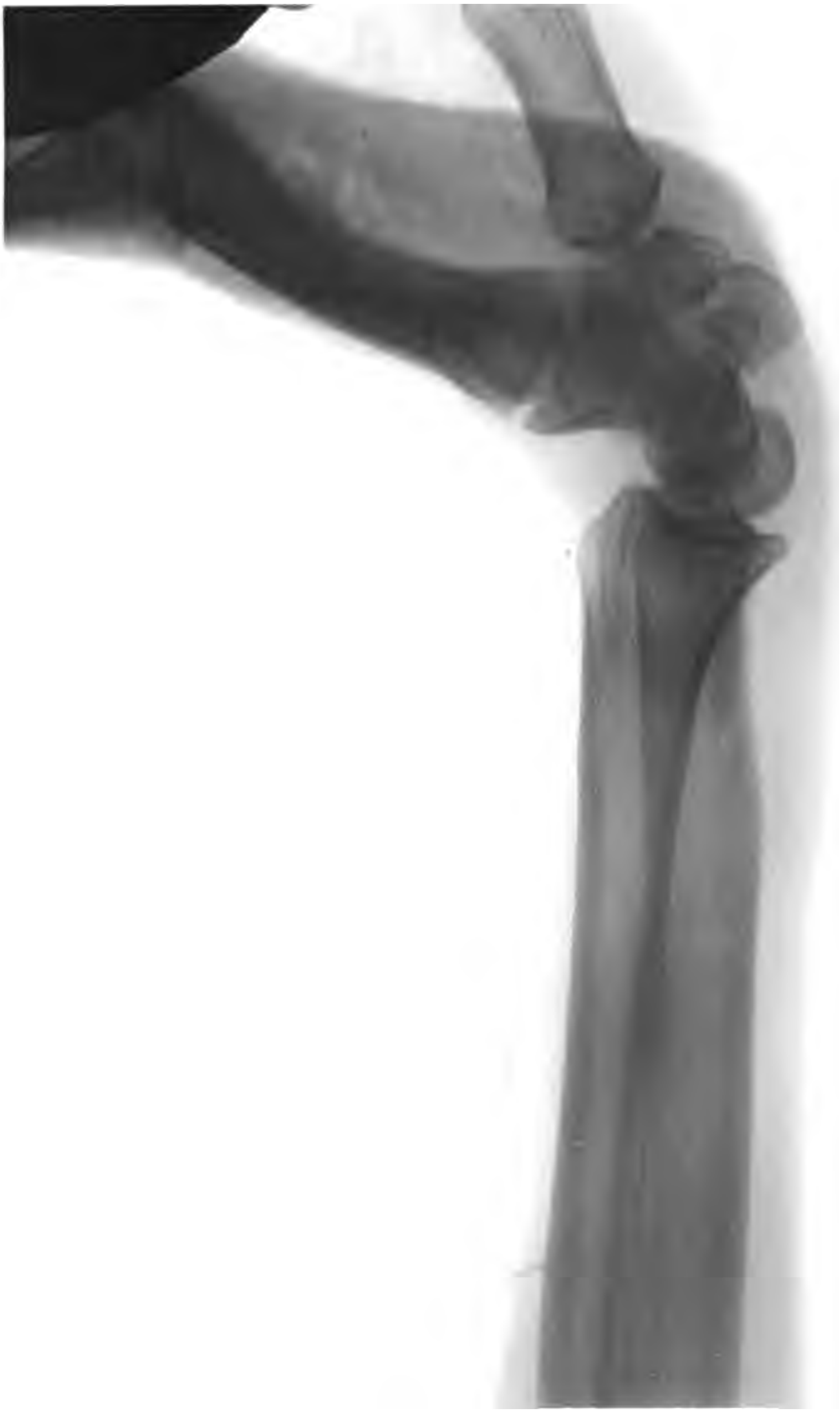

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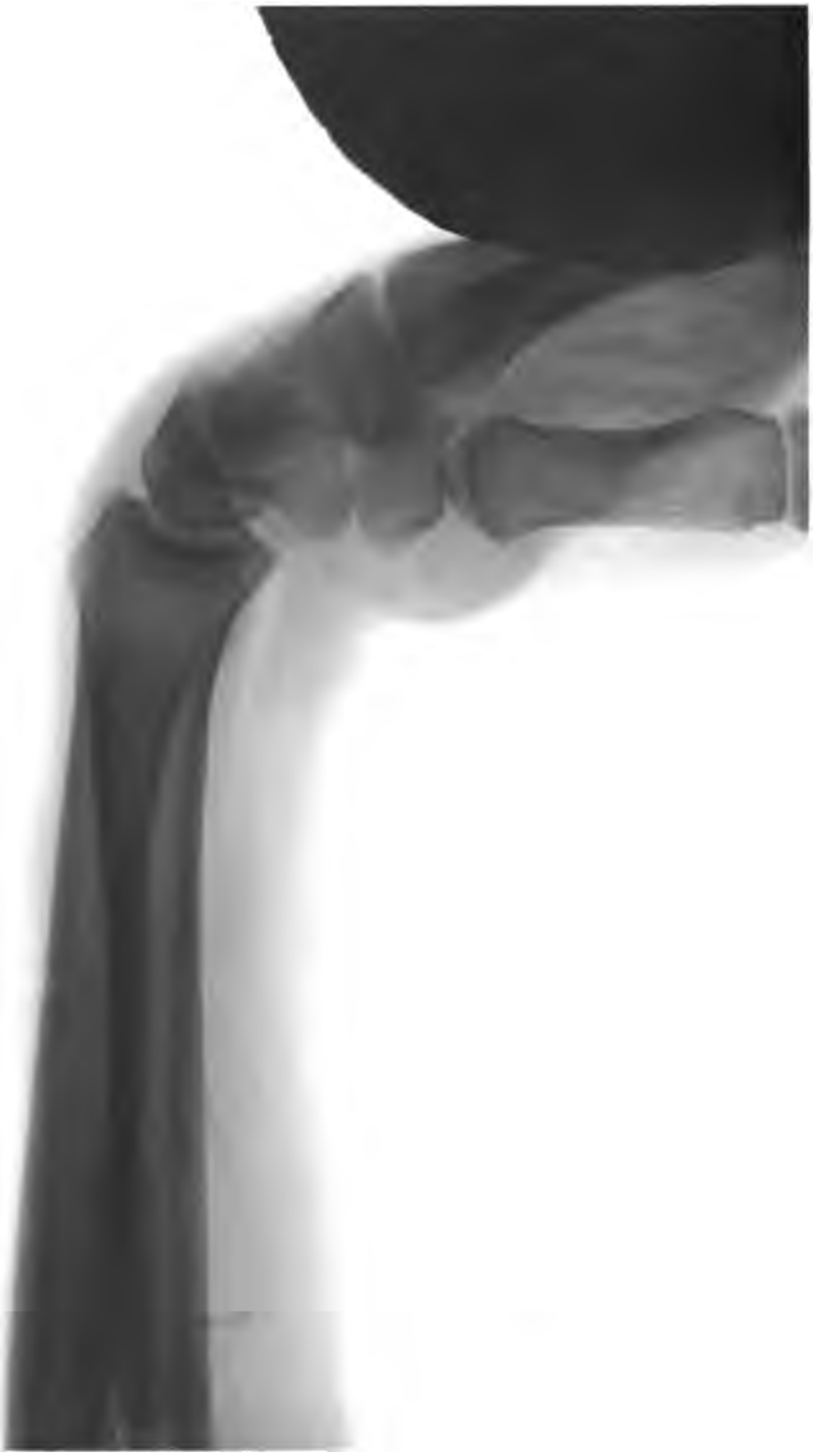

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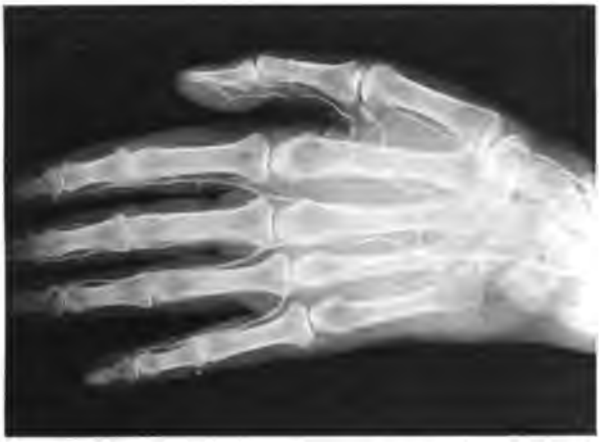

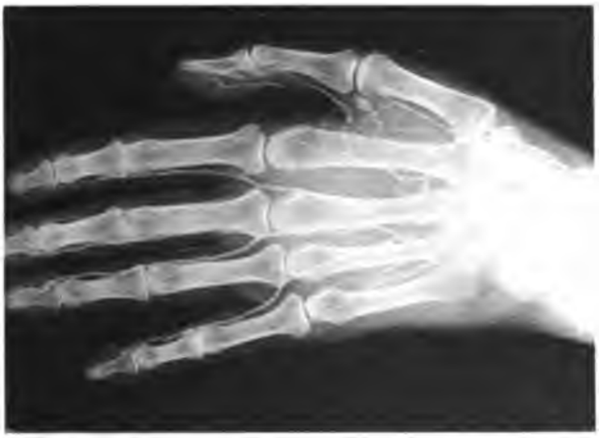

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VOLUME III JULY AND SEPTEMBER, 1898 NOS. 4 AND 5

THE JOURNAL

OF

EXPERIMENTAL MEDICINE

ACUTE INTERSTITIAL NEPHRITIS.

BY W. T. COUNCILMAN, M. D.

(From the Sears Pathological Laboratory of Harvard University.)

PLATES XXXVII AND XXXVIII.

DEFINITION.—An acute inflammation of the kidney characterized by cellular and fluid exudation in the interstitial tissue, accompanied by, but not dependent on, degeneration of the epithelium; the exudation is not purulent in character, and the lesions may be both diffuse and focal.

This condition was first described by Biermer (1). He found the kidneys of a child four and a half years old, who died of scarlet fever, greatly swollen, the surface pale, and on microscopical examination showing intense infiltration of the connective tissue with lymphoid cells. He considers the formation of lymphoid tissue here analogous to that found in typhoid fever. The cellular infiltration is more marked in the pyramids than in the cortex. E. Wagner (2) describes a similar case, in which, however, the enlargement of the kidneys was not so marked. The consistency of the tissue was lax, the capsule easily removed, the cortex grayish-yellow, with punctate hæmorrhages. There were accumulations of lymphoid cells in the interstitial tissue most marked in the immediate vicinity of the glomeruli. In a subsequent article (3) he describes the condition under the name of acute lymphomatous nephritis, and considers it one of the four forms of acute Bright's disease. He finds this form of nephritis most often in scarlet fever, and sometimes in typhoid. The kidneys are greatly enlarged, brittle, the capsule is easily stripped off, the surface is covered with grayish-white elevations which correspond to streaks of cellular, infiltration in the cortex. There are accumulations of round cells varying in size in the interstitial tissue. Some of these cells are smaller than leucocytes, others larger. The most acute cases of scarlatinal nephritis are of the lymphomatous form. In three typical cases which Wagner describes there was pharyngeal diphtheria in addition to scarlatina.

Klebs (4) gives a good description of acute interstitial nephritis. The kidneys are enlarged, of lax consistency, the capsule is easily separated, the surface smooth and paler than normal. On section grayish-white nodules, corresponding to fatty degenerated convoluted tubules, are embedded in a rather transparent, gelatinous, gràyish background. On microscopic examination the tissue between the tubules is dilated and filled with lymphoid cells. The cells come from the vessels. Letzerich (5) describes a kidney of this character in diphtheria, and regards the cells in the interstitial tissue as fungi. Coats (6), in a case of scarlet fever in an adult, finds the kidneys enlarged and similar in appearance to the leukæmic kidney. The enlargement and pallor of the kidneys are due to infiltration of the cortex with round cells. These are packed between the tubules separating them, but have not destroyed the epithelium. The microscopical appearance closely resembles leukæmia. He afterwards describes the cells in the interstitial tissue as due partly to proliferation of the interstitial cells, partly to emigration of leucocytes. The condition is common around the glomeruli in subacute nephritis.

Kelsch (7) regards acute interstitial nephritis as the typical lesion of the kidney in scarlet fever. He describes the cells as being of an embryonic character, closely packed together and often massed around the glomeruli. Klein (8, 9) found seven cases of well-marked interstitial nephritis in twenty-three cases of scarlet fever which he studied histologically. The interstitial cells are lymphoid in character; they vary in size, the nucleus is round, and two nuclei are often found in a single cell. The infiltration seems to take place first around the large vascular trunks at the base of the pyramids, and from here extends into the cortex. These cells compress the tubules, the epithelium desquamates, and the lymphoid cells enter into the tubules. In these cases albumin is often absent, and there are no symptoms pointing to implication of the kidneys. He thinks that cases which die after the ninth or tenth day of the disease will, as a general rule, show more or less well-marked interstitial changes in the kidneys.

Phisalix (10) takes the ground that acute nephritis accompanying various infectious diseases, such as scarlet fever, is not a parenchymatous, but an interstitial process. In such cases but little change may be found in the parenchyma, while the interstitial tissue is dilated and filled with cells. Weigert (11) does not recognize acute interstitial nephritis as one of the forms of acute Bright's disease. In the acute hæmorrhagic form, he describes, in addition to the hæmorrhages and epithelial degeneration, foci of brightly staining round cells in the interstitial tissue. He does not describe the character of the cells further, and considers the foci to be of an inflammatory character and secondary to the degeneration of the epithelium of the tubules and the destruction of the glomeruli. Waller (12) describes interstitial foci as common in post-scarlatinal nephritis. The cells are due to emigration, fibrous tissue may be formed from them, and a contracted kidney may be the final result of the process. Leyden (13) says that acute nephritis in scarlet fever and diphtheria is of a parenchymatous variety, but foci of small-cell infiltration are frequently found between the tubules and around the glomeruli. Bamberger (14) speaks of interstitial foci as common in the primary form of acute nephritis and in nephritis secondary to other diseases. Dunin (15) regards small-cell infiltration as one of the most characteristic changes in scarlatinal nephritis. The infiltration may affect the entire kidney or be limited to certain areas. These areas are most common close beneath the capsule or around the glomeruli. The epithelial changes may be slight, and there is no relation between epithelial degeneration and the interstitial foci. Fischl (16) finds in scarlet fever dilatation of the adventitia of the small arteries with round-cell infiltration extending from this into the tissue. In the cases of glomerulo-nephritis there is infiltration of the interstitial tissue most marked around those glomeruli which were most affected.

Friedländer (17), in his description of scarlatinal nephritis, describes interstitial septic nephritis as one of the three varieties of scarlatinal nephritis. The kidneys in this are large, lax in consistency, and the markings are obscured; the cortex is of a grayish-red opaque color, with foci of hæmorrhage. The epithelium is but little altered, and the interstitial tissue contains numbers of small round cells. This form of nephritis he finds comparatively rare, occurring only twelve times in 229 cases, and is most apt to be seen in those cases of scarlet fever which are accompanied by diphtheritic septic processes. Cornil and Brault (18) find that acute interstitial nephritis is confined to the acute febrile diseases; they have met with it in scarlet fever and other processes. Nauwerck (19,20) describes in the kidneys, in both scarlet fever and diphtheria, foci of small-cell infiltration in the labyrinth and about the small and medium-sized veins. The intertubular capillaries may be occluded by cells derived from proliferation of the endothelium. The cells in the interstitial tissue come from emigration, but may be added to by proliferation of the fixed cells. He finds no relation between these interstitial foci and epithelial degeneration.

Langhans (21) has never found a case similar to Wagner's lymphomatous kidney in scarlet fever, but has seen a slight degree of cellular infiltration in the walls of the small veins and around the glomeruli. Crooke (22) finds distention of the capillaries in the interstitial tissue by lymphoid cells and cells derived from proliferation of the endothelium of the vessels. In many cases foci of interstitial infiltration are found both in the labryinth and close beneath the capsule. Litten (23) says that, apart from fatty degeneration and hæmorrhage, the most common lesion found in the kidney in scarlet fever is the inflammatory round-cell infiltration of the interstitial tissue. Foci of this, consisting of closely packed leucocytes, are scattered through the entire kidney, varying in extent with the intensity of the process.

Oertel (24) in his work on diphtheria describes foci of round-celled infiltration both beneath the capsule and in all parts of the tissue. Cellular infiltration is also seen in the walls of the veins and arteries. There is no connection between the accumulations of cells and desquamation of the epithelium.

Sörenson (25) gives the clinical history and the pathological anatomy of a number of cases of scarlatinal nephritis, and among them of three cases in which the interstitial lesions were well marked. In some of these cases there is a whitish opaque rim around the bases of the pyramids, with streaks extending from this into the cortex. V. Kahlden (26) gives a good description of interstitial nephritis in scarlet fever. He finds the cells in the interstitial tissue of unequal size, some smaller, some larger than leucocytes. In a case of diphtheria he finds numbers of cells in the vessels of the tissue between the cortex and the pyramids. These cells are round, in many cases twice as large as a leucocyte; the protoplasm is homogeneous and transparent. The nuclei are round or sometimes indented, and two nuclei are sometimes seen in a single cell. These cells are more frequently found in the arteries than in the veins. Baginsky and Stamm (27) find in the kidneys of scarlet fever patients who die in the first week of the disease more or less extensive interstitial infiltration with round cells. These cells are either collected into masses simulating lymph nodules or are diffused over the entire cortex.

Bernhard and Felsenthal (28) find in diphtheria, along with degeneration in the epithelium, which they consider the principal change, foci of interstitial infiltration beneath the capsule around the glomeruli and in the upper portion of the pyramids. The veins are dilated and contain numbers of leucocytes. Aufrecht (29) gives the clinical history and pathological anatomy of three cases of scarlatinal nephritis which differ from the ordinary form in their early appearance and intensity. In all three cases there are masses of round cells, chiefly about the glomeruli. The changes in the parenchyma he considers independent of the interstitial lesions. Burmeister (30) describes interstitial foci in the kidneys of rabbits which were produced by the injection of chromate of ammonium into the circulation. There is no especial change in the epithelium of the tubules in the interstitial foci. He found similar interstitial foci around the glomeruli in a case of glomerulo-nephritis in a tuberculous subject. Morse (44) produced similar foci of cellular infiltration of the interstitial tissue of the kidney by injecting filtered cultures of staphylococcus aureus into the circulation of rabbits. Turner (31) makes two varieties of nephritis in scarlet fever, the glomerular and the interstitial hæmorrhagic. In the glomerular type interstitial changes are often seen. Rosenstein (32) says the large interstitial kidney, described by Friedländer, is rare in scarlet fever, although even in cases of short duration, with albuminuria from the beginning, there is evidence of interstitial proliferation with focal accumulations of cells around the vessels.

Summing up these descriptions, I find that acute interstitial lesions of the kidneys have been considered as common in scarlet fever, and are regarded by some authors as constituting the most frequent pathological alteration of the kidneys in this disease. This has also been described in diphtheria and in other infectious diseases. The cellular infiltration of the interstitial tissue may be general throughout the kidney, with more intense foci in the cortex. In these cases the kidney is greatly enlarged, in some cases twice the normal size; it is of a lax consistence, and on section is grayish and opaque. In other cases the infiltration appears as circumscribed foci either immediately beneath the capsule or around the glomeruli or at the bases of the pyramids. The cells in the interstitial tissue are almost universally described as lymphocytes, in several cases their large size and the presence of two nuclei in a single cell are mentioned. Cell accumulations have also been described around the large vessels and infiltrating their walls. Von Kahlden describes in one case accumulations of large cells in the vessels in the pyramids. The cells have been generally considered as coming from the vessels by emigration. Almost all observers state that there does not seem to be any relation between foci of interstitial cellular infiltration and epithelial degeneration. There is general agreement that in scarlet fever the interstitial form of nephritis is most often found when the nephritis develops early in the disease, and is most common in the so-called septic cases. Many of the cases in which the most typical interstitial lesions were found are described as having also diphtheritic pharyngitis. No evidence has been given by cultures that in these cases there was mixed infection with diphtheria.

The text-books on pathology do not devote much space to this condition. Most of the authors do not recognize acute interstitial nephritis as a special type of the disease. Others speak of foci of interstitial cellular infiltration as occurring frequently in acute and chronic nephritis. In many cases the cells in the interstitial tissue are regarded as pus-cells and the condition is confounded with acute suppurative nephritis. Ziegler (33) has a very good description of the minor degrees of the process. In speaking of inflammatory œdema of the kidney he says, " the changes in the vascular stroma may take the form of circumscribed cellular infiltrations of the interlobular and circum-glomerular tissue, a condition of acute disseminated interstitial nephritis. The cellular infiltration makes its appearance in the neighborhood of the stellate and interlobular veins, and is usually so intense in these parts that in stained preparations the infiltrated patches are easily discernible under a low power of the microscope. The cellular aggregations are most numerous in the outer zone of the cortex and in the boundary zone between the cortex and medulla, the middle part of the cortex being seldom much affected." He says nothing of the character or origin of the cells, and thinks the condition may run a subacute course, gradually passing into the parenchymatous type.

The best description of the gross and microscopic condition in such kidneys is given by Orth (34). He says:

> " It is rare that œdema is the most essential inflammatory change in the interstitial tissue. I have often seen kidneys in which the tubules and glomeruli were normal and in which the only change consisted in a

small-celled infiltration of the interstitial tissue. Such cases have been described as acute interstitial nephritis, as productive nephritis, as granulating nephritis, and as lymphomatous nephritis. These kidneys are more or less enlarged and softer; the surface is mottled, of a grayish-red or grayish-white color, the markings cannot be distinguished. Such kidneys can be distinguished from those of acute parenchymatous nephritis by the absence of the markings and the cloudiness of the cortex. Microscopically there is an irregular distribution of cells between the urinary tubules. These cells have a round vesicular nucleus, they are not similar to emigrated leucocytes, and I regarded them as due to growth of the connective-tissue cells, although their frequent relation to the small veins would rather speak in favor of emigration."

I have previously (35) reported eight cases of this lesion in the kidney; three of these were from scarlet fever; two from diphtheria; one followed a criminal abortion, and two were of unknown origin. In all the cellular infiltration of the interstitial tissue was the most marked, and in some cases the only change. Since then I have had the opportunity of examining microscopically the kidneys from a great many cases of diphtheria, scarlet fever, and other acute infectious diseases, and in the routine microscopic examination have found cases which showed every degree of alteration and most of which would have been overlooked without microscopic examination. The result of the further study of this important lesion in the kidney has thrown some light on the subject, and certainly caused me materially to change my views as to the origin of the cells in the interstitial tissue. In my previous communication I regarded the interstitial cells as derived from proliferation of the fixed cells of the tissue, particularly from the endothelium of the blood-vessels.

MACROSCOPIC CHANGES.

In the most marked cases the kidneys are greatly enlarged; in one case of diphtheria and measles in a child aged two years the combined weight of the kidneys was 480 grammes. In another child aged eight years, with scarlet fever, the kidneys weighed 400 grammes; in two other cases of diphtheria, one in a child of five, the other in a child of two and a half years, the kidneys weighed 250 and 225 grammes. This extreme size, which may be more than three times the normal, as in the first case mentioned, is rare. In most cases the kidneys were but slightly if at all enlarged. The appearance varies. In the most marked cases the kidney presents an appearance which can be mistaken for nothing else unless it be the leukæmic kidney. The capsule is distended, thin and easily removed, often separating spontaneously on section of the kidney. The fœtal markings are less distinct and often obliterated. The surface is pale, of a peculiar grayish-opaque color, somewhat resembling the amyloid kidney, mottled, with irregular, more hyperæmic areas; the stellate veins of the surface are injected, often showing small punctate ecchymoses around them. In some cases the surface is irregular, due to the projection of small irregular nodules which are more opaque than the surrounding tissue.

On section of such kidneys the normal markings of the cortex are obliterated, and the contrast between pyramids and cortex is less distinct. The great increase in size is principally in the cortex, which may be three or more times thicker than normal. The glomeruli are not injected, and cannot be distinguished. The general color of the cut surface is grayish and opaque, with scattered areas of injection, and a few small scattered ecchymoses. Corresponding to the elevated areas on the surface, there are often areas more opaque than the general cut surface, which extend in lines from the pyramids through the cortex. At the bases of the pyramids the same opacity of the tissue is seen. The tissue of the kidney is soft, lax, and easily broken. It is very moist, and an opaque milky fluid may be pressed out, and often flows from the cut surface. On scraping the tissue with a knife a milky fluid with small granular masses may be removed. In the less marked cases there is usually some degree of mottling, the cut surface is moist, and there are fine opaque lines or points. In many cases the changes are most evident at the bases of the pyramids and marked by a swelling and greater opacity of the tissue here. A very marked degree of change, as shown microscopically, may be present without producing macroscopic differences in the appearance of the kidney.

MICROSCOPIC CHANGES.

Sections of the fresh kidneys, to determine the presence of fatty degeneration, showed this change to be generally present, though in slight degree. Fat was found in the epithelium of the tubules, and principally in the smaller collecting tubules. In a few cases foci of more intense fatty degeneration were found in the convoluted tubules. Occasionally a slight amount of fat was found in the interstitial tissue, but not in the cells infiltrating this.

For microscopic examination thin sections of the tissue were hardened in Zenker's fluid, in corrosive sublimate, in alcohol, and in Flemming's solution. In general the best results are obtained after hardening in Zenker's fluid, and this was used in all of the routine examinations. The other solutions were only used in special cases and where the lesions were evident on macroscopic examination. Various stains were used, the most satisfactory results being obtained by staining deeply with eosin, followed by Unna's alkaline methylene-blue solution.

In the most marked cases examination with low power shows the tubules to be widely separated and an intense cellular infiltration of the interstitial tissue. This is marked in all parts, but is more intense in certain areas. These are most often situated beneath the capsule (Plate XXXVII, Fig. 1) and at the bases of the pyramids, and from these places the infiltration sometimes extends downward or upward. In the middle of the cortex there are also smaller areas of intense infiltration which seem not to be connected either with the areas beneath the capsule or with those at the base of the pyramids. In these areas the interstitial cells are closely packed together, with no intervals between them. Where the infiltration is less intense, the cells lie singly in the tissue. Around all the glomeruli, but outside of the capsules, there seems to be more marked infiltration than elsewhere, save in the areas mentioned. In the pyramids the infiltration is often as intense as in the cortex, extending down to the pelvis.

With high power (Plate XXXVII, Fig. 2) the cells in the interstitial tissue present a striking appearance. Most, and in some cases all of them, are of the same character. They vary somewhat in size and in shape. They are generally much larger than the polymorphonuclear leucocytes, and may be from two to four times the diameter of a red blood-corpuscle. Their shape varies; the single cells not in contact with one another may be round; but where they are packed together the shape is influenced by the mutual pressure. The nuclei present some variation in size, they are never vesicular, the periphery stains deeply, and there are intensely stained points of variable size in the interior. The stained periphery often projects at certain points into the interior of the nucleus, and in most of the nuclei an intranuclear network connecting the brightly stained points within the nucleus and the points on the nuclear rim can be made out. The general body of the nucleus stains deeply. In most cases there is but a single nucleus in a cell. In others two or even three may be present. The nucleus occupies a very characteristic position. It is eccentric, and when the cells are elongated it lies usually at one of the ends. This eccentric position of the nucleus is sometimes so marked that it apparently projects from the protoplasm.

The amount of protoplasm around the nucleus varies. Most of the cells have a large amount, while in others the nuclei are found with but little surrounding them. The protoplasm is dense and often finely granular. In some of the cells the granulation is much more evident than in others. Even in the cells lying singly in the tissues the contour is not always sharp and round. In many cases both the cells here and in the masses have an irregular outline due to the protrusion of elongated and often knob-like masses of protoplasm. These present every resemblance in shape to leucocytes fixed while in emigration, and there is little doubt that these irregularities of form are due to amœboid movements. There is no appearance of a membrane around any of these cells. Their most characteristic feature is the staining of the protoplasm. With most of the nuclear stains used singly the protoplasm stains faintly, but more intensely than that of the epithelial cells. With strongly alkaline methylene-blue the protoplasm stains deeply, and the blue color is preserved after counter-staining with eosin. A faint bluish tint, giving with the eosin a lilac tint, is often seen in the protoplasm of the epithelial cells, but in the interstitial cells the color is a deep blue. The cells can be instantly distinguished in a section even with a low power by their characteristic color. Nuclear figures are very numerous. As many as three or four are often seen in the field of an oil immersion. The cells in which the nuclear figures are found are large and have an abundant and distinctly granular protoplasm, the granules staining a deep blue.

These cells are found in the interstitial tissue. In all of the sections examined I have never found one of them within a tubule. In the interstitial tissue they lie loosely in the spaces of the tissue, and a few are constantly found in the blood-vessels. They are not phagocytic; neither leucocytes, red blood-corpuscles, nor detritus of other cells are found inclosed in them.

In the very marked cases, in which the kidneys are soft and enlarged, other cells are constantly found in varying numbers in the interstitial tissues. The most numerous are cells corresponding to the lymphoid cells (Plate XXXVII, Fig. 2); the nuclei of these stain very much as do the nuclei of the large cells, and every gradation can be seen between the naked nuclei of the lymphoid cells and the large cells. Polynuclear leucocytes are usually present in varying numbers, depending on the degree of degeneration in the epithelium. In a few cases, especially where the lesions are very marked and the tissue is disintegrated, I have also found cells of the epithelioid type in the interstitial tissue. These cells are, as a rule, larger, the protoplasm is finely granular, staining with eosin, the nucleus is less intensely stained than are the nuclei of the interstitial cells, and clearly vesicular. They can be easily distinguished from the blue-staining cells and there is no transition between them. These epithelioid cells, which are probably derived from proliferation of the tissue cells, are eminently phagocytic. I have found red blood-corpuscles, leucocytes, and in some cases the blue-stained cells inclosed within them. Nuclear figures are occasionally seen in the epithelioid cells, but never to the extent observed in the blue cells.

The epithelium shows various degrees of degeneration, and in places the tubules seemed to be actually destroyed.

The tubules are separated by wide areas of interstitial cellular infiltration, and often no tubules can be found in comparatively large areas. The slightest change found in the epithelium is a marked degree of cloudy swelling. In places the cells contain globular masses of hyaline, and the lumen of the tubule may be filled with the same material. In other cases the cells are swollen, vacuolated, and the greater part of the protoplasm may be lost, leaving only a small granular mass about the pale and rather shrunken nucleus. In places the tubules may contain desquamated epithelium or granular masses derived from it. The number of leucocytes present seems to depend upon the degree of destruction of the epithelium; they may be seen in the interstitial tissue, in the epithelial cells, and in the lumina of the tubules. The glomeruli usually show no changes, although cases of typical glomerulo-nephritis may be met with in which acute interstitial nephritis is present as a complication. Granular coagulated material may be present in the capsular space, but even this is often absent.

In the less marked cases (Plate XXXVII, Fig. 1) the general diffuse interstitial infiltration is absent. A few scattered blue-staining cells may be found, but the lesions are eminently focal. In some cases the foci are so numerous that on holding a section to the light they appear as scattered blue specks or lines (Plate XXXVIII, Fig. 3). Most of these areas, when observed with the naked eye or a very low power, have their longitudinal axes in the direction of that of the pyramids. In these foci the cells in the interstitial tissue are in great numbers, but not so numerous as in the foci in the most marked cases. These foci are principally found in three places in the tissue. As a rule, they are most numerous and largest at the bases of the pyramids, and from this point they may extend upward in the pyramidal extensions. Occasionally they are in large masses around the interlobular vessels. Next in frequency the foci are found beneath the capsule around the large branches of the stellate veins, which are often greatly dilated. Smaller foci may be found at various places throughout the cortex, but in the cortex they are most frequent around the glomeruli. The number of foci around the glomeruli varies greatly in different cases. In some there are cell masses around practically every glomerulus, in others only scattered glomeruli are affected. I have never found a case in which the interstitial areas were confined to the tissue around the glomeruli, but there were numerous cases in which the infiltration was confined to the bases of the pyramids or to the subcapsular cortex.

These focal areas seem to stand in no relation to focal degeneration of the epithelium. In all cases degeneration of the epithelium is present. Where the infiltration is most intense there is apt to be extensive degeneration and even necrosis of the epithelium of the tubules, but this is probably secondary, and due to compression of the tubules and vessels. In these larger areas there are usually numbers of pus cells which may be found within the tubules (Plate XXXVII, Fig. 2). In the smaller areas no evidence of greater degeneration of the epithelium can be found than elsewhere in the kidney, and a high degree of degeneration may be found without any interstitial changes.

These changes in the interstitial tissue are always accompanied by changes in the vessels (Plate XXXVIII, Figs. 4, 5, 6, 7). The interstitial cells are found in the vessels as well as in the tissue, and they may be so crowded in these and in the surrounding tissue that it is difficult to distinguish the vessels. Most of the cells in the vessels have the same character and the same peculiarity of staining as the cells outside. Nuclear figures (Plate XXXVIII, Figs. 5 and 7) are found in these cells, but not in such numbers as in the cells in the interstitial tissue. There are few polynuclear leucocytes among them. The numbers of cells in the vessels are not always proportionate to the numbers in the interstitial tissue. Vessels blocked with cells may show but slight interstitial change, and occasionally interstitial foci are found where but few cells can be made out in the vessels.

In making a systematic study of the kidneys in a large number of cases of acute nephritis special attention was given to the presence of these cells in the vessels. The vessels in which the greatest number were found are always the branches of small veins in the upper part of the pyramid. In a large number of cases such cell accumulations were found in the vessels without any change in the interstitial tissue and without any more than the ordinary degeneration of the epithelium. In some cases all of the veins in this part of the kidney were so blocked up with cells that on holding a stained specimen to the light there was a decided blue color of the upper part of the pyramid. Such cell collections were occasionally seen in the stellate veins of the cortex and elsewhere, but in many cases they were confined to the veins of the pyramids. Most of these cells in the veins were of the same character as those in the interstitial tissue. The main difference between the cells in the vessels and those in the tissue was that the vessel cells were round and regular in shape. These cell accumulations in the vessels without interstitial lesions were found in 16 out of 103 cases of pure diphtheria; in 1 case out of 19 in pure scarlet fever; in 3 out of 23 in diphtheria and scarlet fever; in 2 cases of diphtheria and measles; in 1 case of pyelo-nephritis, and in 1 case of acute endocarditis; in 24 cases in all.

There can be but one source for the cells in the interstitial tissue; they must come from the blood-vessels. In the first place, there are no cells in the tissue outside of the blood-vessels which could give origin to them. Practically the only cells which we have in the interstitial tissue of the kidney outside of the small collections of lymphoid cells, sometimes seen around the larger veins, are the cells of the blood and lymphatic vessels. It is probable that there are cells belonging to the connective tissue, but it is difficult to demonstrate their presence. These cells in the most advanced cases, in which there is actual destruction of the tissue, do proliferate and mingle with the other cells in the interstitial tissue. The resulting cells are the epithelioid cells, and can be distinguished by the nucleus, the staining of the protoplasm and their marked phagocytic properties, from the interstitial cells. Cells similar to the interstitial cells are found in the blood-vessels in the interstitial cases and in cases in which there are no interstitial lesions. It is impossible to see the irregular shapes and protrusions of the protoplasm of many of the cells in the interstitial tissue without being convinced of their amœboid properties. In a few cases similar changes of form were seen in the cells within the vessels, and in several instances such cells were found in the act of emigration, part of the cell being within and part without the vessel (Plate XXXVIII, Fig. 10). The cells are principally confined to the vessels in the places I have mentioned. A few may be found in the larger veins, and they are occasionally seen in the vessels of the glomerulus.

The part which these cells play in pathology has received but little attention. Similar collections are most frequently described as small-celled infiltration, and no attention has been directed to their character and source. Unna was the first one who separated out from the cell collections in the interstitial tissue certain cells marked by a peculiar stain of the protoplasm, to which he gave the name plasma cells. Since then they have received considerable attention from the dermatologists and but little from pathologists. I consider the cells I have described in the interstitial tissue identical with Unna's plasma cells. Unna (36) describes in lupus cells, whose protoplasm stained deeply with alkaline methylene-blue, and which do not decolorize as the other cells. He considers them identical with the cells described by Waldeyer as plasma cells, and gives them the same name. As these cells appear in the tissue the connective-tissue cells disappear, and he supposes that they are derived from the connective-tissue cells. In his first article on the subject Unna supposes that these cells were peculiar to the tuberculous process. He subsequently (37) describes the cells more fully, modifying his first description in several particulars. In this article he considers them as greatly hypertrophied connective-tissue cells. The blue staining is due to the presence of fine granules imbedded in the substance of the cell. He finds that they play an important part in the pathology of skin diseases, and that certain new formations in the skin, to which he gives the name plasmomata, may be composed of them. The tumor cells in the round-cell sarcoma are derived exclusively from these cells, and they form the chief part of the cellular infiltration around other tumors. They are exclusively a pathological product, and are never found in embryonic or in normal tissues.

Jadassohn (38), while confirming the presence in the tissues of the cells described by Unna, denies that they are to be differentiated from the other cells of the tissue solely by Unna's method of staining. He finds them in various inflammatory affections of the skin, and thinks they may be present in any form of acute inflammation with the exception of the suppurative. While he is uncertain about their origin, he denies that they are related to the connective-tissue cells.

Waldeyer at first believed that the cells described by Unna were identical with his plasma cells, but he afterward (39), in an article on the cells of the connective tissue, declares that the latter belong to the group of Mast-cells, which are different from the cells described by Unna. He does not think the word plasma cell should be used to designate any of the connective-tissue cells.

Marschalkó (40) has written an important article bearing on the presence and origin of these cells. He finds them not only in various tumors and chronic inflammatory conditions, but also in acute inflammation produced by the injection of carbolic acid into the liver, and in acute suppurative inflammations produced in the subcutaneous connective tissue. They appear in the tissue within twenty-four hours, and are derived from the lymphoid cells of the blood. These accumulate in the vessels in the periphery of the inflamed area, pass through the walls, and change into plasma cells in the tissues. He lays more stress on the morphology of these cells, the character of the protoplasm and nucleus, and the constant eccentric position of the latter, than on any peculiarity of staining. He finds them in the vessels of the liver at a distance from the inflammatory focus and in the vessels of the spleen. He also finds them in small numbers in the spleen, lymph-glands, and bone-marrow. He makes the very important observation that after the injection of tuberculin their numbers in the vessels of the spleen are greatly increased.

Hodara (41), working under the direction of Darrier, took up again the question of the presence of plasma cells in the normal tissues. He does not find them in the bone-marrow, lymph-glands, or spleen, in the normal embryo, child, or adult. He thinks that Marschalkó confounds the plasma cells with certain large mononuclear leucocytes, for which he proposes the name polyeidocytes, the name having reference to the variability of form which characterizes these cells. In some instances these cells may so resemble the plasma cells in size, form, character, and staining of the nucleus and protoplasm, as to be indistinguishable from them. In general the difference between the polyeidocyte and the plasma cell consists in the great irregularity of form of the polyeidocyte and the variability in form, size, and staining of its nucleus. The only other article of importance concerning the plasma cells is that of Justi (42), who takes up the part which they play in the formation of the granulation tissue. Justi has included among the plasma cells large cells in the granulation tissue containing several nuclei. These cells are characterized as much by the character of the nucleus and protoplasm as by their manner of staining. Justi places the plasma cells among the leucocytes, and thinks they are derived from the lymphocytes.

It is perfectly evident that the cells we have described in the interstitial tissue, and many of those in the cell accumulations in the blood-vessels of the kidney, are the plasma cells of Unna. As to their origin, I hold the same opinion as Marschalkó, and they are derived from the lymphocytes. In the kidney they enter into the interstitial tissue by emigration from the blood-vessels. They may emigrate from the vessels as plasma cells, or they may be formed from emigrated lymphoid cells. They have been seen in the act of emigration, and the shapes of many of the cells in the interstitial tissue can leave no doubt as to their amœboid character. A great part of those in the interstitial tissue come from proliferation. It is difficult to understand why previous observers of these cells should have been so uncertain as to their mitotic division, and should have found it necessary to resort to the very uncertain theory of multiplication by direct division of the nucleus. Nuclear figures are as numerous in the interstitial cells, and as definite considering their size, as will be found in a rapidly growing carcinoma. Certainly there is no difficulty in explaining the numbers of these cells met with in the kidney by emigration and proliferation. Nuclear figures are also found in the cells within the blood-vessels, but not to the same extent as in the interstitial tissue.

With few exceptions, we have examined in every case of diphtheria and scarlet fever sections hardened and stained in the same way not only from local lesions, but from the liver, spleen, lungs, lymph-glands, and bone-marrow. A detailed account of these examinations will appear later. It will be sufficient to say here that in nearly every case of both diphtheria and scarlet fever, but particularly diphtheria, both those in which interstitial lesions in the kidney were marked and where they were absent, great numbers of plasma cells are found in the spleen and bone-marrow. They are also found in the lungs, liver, capillaries, and lymph-glands. The principal seat of their formation seems undoubtedly to be the spleen, where they are formed from the lymphoid cells. Sections of the spleen in some cases show nearly the whole of every Malpighian body converted into plasma cells. Groups of them were also found in the pulp of the spleen and in the vessels. In all these places there are numerous nuclear figures. It is not probable, as Unna assumes, that direct nuclear division ever takes place in these cells. In the bone-marrow, which is red in nearly every case, numerous groups of plasma cells are also found. In the lungs they are found in great numbers in the blood-vessels, but especially in the connective tissue of the lung around the bronchi and blood-vessels. In places they play an important part in the cellular exudation in the focal pneumonia, but are found principally in the vessels and interstitial tissue, rarely in the alveolar exudation. In the lymph-glands, save in those in the immediate vicinity of the throat lesions, they are much less numerous than in the spleen and bone-marrow. In the liver they are in the capillaries, and probably come there from the spleen. Sections from the larger blood-vessels containing blood, and of decolorized blood-clot from the heart, are not so satisfactory for examination as the tissues, but they are found here in small numbers.

Investigations are now in progress to determine the presence and numbers of these cells in the peripheral circulation in cases similar to those in which they are found in the tissues.

With regard to the presence of the plasma cells in normal tissues, it is remarkable that those investigators who have taken up this question should have left out of consideration the organ in which they are always present in enormous numbers. In the alimentary canal, from the stomach to the anus, all the cells of the tissue between the epithelium and the muscularis mucosa are practically plasma cells. They are comparatively rare in the follicles in this region.

In the tissues examined for fatty degeneration, both in fresh sections and after Flemming, there is no fat found in the plasma cells. Evidence of cell necrosis, as shown by nuclear detritus, is not found in them. The only evidence of degeneration which may be present in the plasma cells, both in those in the interstitial tissue and in those in the blood-vessels (Plate XXXVIII, Fig. 8), consists in the presence of vacuoles in the cells, or hyaline droplets which occupy the same position as to the vacuoles, and which often stain homogeneously with methylene-blue.

We are led to the belief that the plasma cells have their origin in the lymphoid cells from the similarity of their nuclei to those of the lymphoid cells, and from the presence of transitional forms between the lymphoid and plasma cells, as shown by the gradual accumulation of protoplasm of the same character as that of the plasma cell around the nucleus. This does not take place evenly around the nucleus, but always at one side. The constant eccentric position of the nucleus of the plasma cell is one of its marked characteristics.

In the examination of the kidneys of the autopsies in the last two and a half years I have found 42 cases of acute infectious diseases in which acute interstitial nephritis was present. The service in the hospital is an acute one, and there are comparatively few autopsies on chronic cases. All of the cases in which interstitial infiltration with plasma cells was found are included in the 42 cases; those in which only lymphoid cells were found around the large interlobular veins are not included. The majority of the interstitial cases come from the acute infectious diseases of children, which make up a large part of our autopsy material. In 103 cases of pure diphtheria a varying extent of interstitial change was found in 24. In 20 cases of pure scarlet fever interstitial lesions were found in 5. In 23 cases of mixed infection of diphtheria with scarlet fever, interstitial nephritis was found in 5, and in 2 out of 5 cases of diphtheria and measles. The other diseases in which the lesion was found were measles and whooping-cough, 1; empyæma, with subsequent diphtheria infection, 1;

lobar pneumonia and pericarditis, 1; epidemic cerebro-spinal meningitis, 1; lobar pneumonia, 1; acute glomerulo-nephritis, 1; acute endocarditis following abortion, 1.

Acute interstitial nephritis is far more common in the infectious diseases of children, and is occasionally seen in other acute infectious diseases. This fact and the focal character of the lesions suggest at once the agency of bacteria. The general diffuse degeneration of the epithelium which was so constantly found is evidently to be referred to the action of some soluble toxic substance. The results of the bacteriological investigation, both by microscopic examination of sections stained for bacteria and by cultures of the organs made at the autopsies, have not borne out the bacteriological theory. In all cases cultures were made at the autopsies from the primary lesions of the disease, whatever its character—from the blood, lungs, liver, kidneys, and spleen. The results of the cultures from the kidneys in the 42 cases of interstitial nephritis were as follows: In 24 cases of pure diphtheria the kidney was sterile in 6. In 11 the colon bacillus was found; in 1 case the staphylococcus aureus; in 5 cases the streptococcus pyogenes; in 8 the diphtheria bacillus, and in 1 the bacillus fœtidus. In the 5 cases of pure scarlet fever the colon bacillus was found in 2, the streptococcus in 3, and the staphylococcus in 1. In the 8 cases of mixed infection of diphtheria with scarlet fever or measles 2 were sterile; in 3 the streptococcus, and in 3 the colon bacillus was found. In the other 6 cases the colon bacillus was found in 3; the staphylococcus aureus in 2; the streptococcus pyogenes in 4; the pneumococcus in 1, and 1 case was sterile. I have included the colon bacillus in the result of the bacteriological examination, although I am not inclined to regard the presence of this as determined by cultures made at the autopsy as a matter of much etiological value. The result of the bacteriological examination of the kidneys in the same diseases, but in which interstitial lesions were not present in the kidneys, have shown the same results. The kidneys were sterile or the same organisms were present in about the same proportion. It is obvious from this that we cannot lay any weight on the presence of bacteria in the kidneys as a causative factor in the acute interstitial lesion.

In seeking an explanation of the focal character of these lesions their situation must be taken into account. They are most marked: First, in the boundary zone of the pyramids; secondly, beneath the capsule; and thirdly, around the glomeruli. There can be but two explanations for the presence of these foci. Cells are brought to the foci by the vessels, they emigrate from the vessels into the interstitial tissue, but probably the greater part of those in the interstitial tissue come from proliferation. Either there must be some physical condition in the circulation which may account for their accumulation in the vessels, or there must be some substance in the tissues which exerts a positive chemotaxis for just these cells, and which causes both their accumulation in the vessels and their emigration into the tissues. There is much which seems on purely physical grounds to favor the accumulation of cells in the long veins of the pyramids. These veins are long, they are numerous, and the circulation in them is slow. It is possible that the large cells in the circulating blood might be mechanically retained in these vessels. Weight is given to such a supposition by the fact that even small substances in the circulating blood may be mechanically retained here.

The hæmatogenous abscesses of the kidney, both those produced experimentally and those seen in man, are more apt to be formed in the upper part of the pyramid than elsewhere in the kidney. The organisms for the most part pass through the capillaries of the cortex; they become attached to the walls of the veins, and increase there, finally occluding the vessel and producing necrosis in the surrounding tissue. The presence of large numbers of these cells without any emigration speaks in favor of such a mechanical theory. The same would hold true, though to a less extent, in regard to the cell accumulations in the stellate veins. Occasionally the cells are seen here without interstitial lesions, but to nothing like the same extent as in the pyramidal vessels. Nuclear figures are found in the cells within the veins. The cells can pass through the vessels into the interstitial tissue, and the same conditions which led to their production and proliferation in the first place could cause them to proliferate in the interstitial tissue. Tumor cells can act in this way, and there is certainly some temptation to regard the interstitial foci very much in the same light as tumor metastases.

Such a mechanical theory fails to explain the interstitial foci around the glomeruli. Occasionally a few cells may be found in the vessels of the glomeruli (Plate XXXVIII, Fig. 9) without any interstitial lesions. Not only do periglomerular foci speak against the mechanical theory, but these cells are not found to the same extent in the vessels elsewhere, even in organs where the conditions of the circulation would seem to favor their presence. A few are almost constantly found in the capillaries of the liver, but their production in the spleen would explain this. They are found in the vessels of the spleen and bone-marrow, and in both of these places they are evidently produced. On the other hand, assuming that their presence in the kidney is due to some substance which exerts a positive chemotaxis for them, there are equal difficulties in an explanation. They are not attracted by simple degeneration and necrosis of tissue, as are the polynuclear leucocytes, and are not simply secondary to epithelial degeneration. Dr. Morse in a study of cantharadin poisoning (not yet published) has shown most extensive degenerative lesions, and even necrosis, without any change in the interstitial tissue and without leucocytes.

Ribbert (43) has recently published an interesting article in which he endeavors to explain the focal accumulation of cells in the kidney. In this article he calls attention to the locality of the foci of cellular infiltration and atrophy. He finds in chronic nephritis the lesions in the same situations which I find in the acute interstitial process. Ribbert explains this localization by the degeneration produced in the irregular tubules (Schaltstücke) by the concentrated toxic urine, and by the toxic urine passing directly through the thin capsules of the glomeruli. The pyramidal lesions are due in part to the toxic action of the urine in the collecting tubules, and in part to toxic substances which have passed into the tissue, passing along the lymph spaces to the large lymphatics in the boundary zone. It is possible that the general degeneration of the epithelium which we find in these cases may be due either to the toxic qualities of the urine or of the blood. The interstitial lesion may be due to some substance excreted by the kidney, the foci being determined either by the action of this material where it becomes more concentrated, or where it escapes into the tissue, or the paths which it takes in the tissue. It is possible that the physical conditions of the circulation in the kidney may play a part, and possibly an important part, in determining the foci.

But little is gained from the clinical study of these cases. There was albumin in the urine, as there is in all cases of diphtheria. In none of the cases was there marked diminution in the amount of urine, and in none was there œdema. There was apparently nothing in the clinical history of the cases which called especial attention to the condition of the kidneys. In the literature of the disease there are some cases in which the clinical history is given. In Biermer's case, the disease being scarlet fever, the renal symptoms began two weeks before death, with marked diminution in the amount of urine, and dropsy followed by uræmia. In 3 cases of acute lymphomatous interstitial kidney in scarlet fever which Wagner reports, 1 died on the tenth and 2 on the seventeenth day of the disease. All 3 of the cases had pharyngeal diphtheria. Klein found the condition most frequently in those patients with scarlet fever who died after the ninth day of the disease. Friedländer found the lesions, which he describes as interstitial septic nephritis, in those cases of scarlet fever which are accompanied by severe diphtheritic septic processes.

The disease has a rapid course, and normal urine may be seen even twenty-four hours before death. Usually there is no œdema. It is probable that the 12 cases which Friedländer described, and which were from 229 autopsies on scarlet fever, represented marked types of the disease in which the lesions were evident macroscopically. Had he made systematic microscopic examinations he must have found minor degrees of similar lesions much more frequently. Sörensen gives the clinical histories of 3 cases. He also says the interstitial form is most common in cases of severe diphtheritic septic scarlet fever, and appears early in the disease. In one of his cases there was slight œdema and ascites.

In addition to these 42 cases, in which the interstitial lesions of the kidney were due to a general disease, 3 were found in which the interstitial process was due to the local action of bacteria.

The first case was one of ascending ureteritis and pyelonephritis. The kidneys were enlarged, weighing 390 grammes (small, slender woman), injected, somewhat œdematous, and on section whitish-yellow streaks in the cortex extended from the bases of the pyramids toward the capsule. The tissues in these areas of infiltration were not softened. Cultures from these areas gave colon bacilli and a few colonies of streptococci. On microscopic examination of sections in some of the larger areas there was a mass composed of necrotic tissue and filled with nuclear detritus. There were small areas where the necrotic tissue seemed to have been composed of pus cells, but for the most part the remains of the cells and nuclei seemed to have come from large cells. In the periphery of the larger foci, and in the whole extent of the smaller, there was intense infiltration of the tissue with plasma cells. Sections from the ureter and pelvis of the kidney showed slight superficial necrosis, comparatively few pus cells, and great numbers of plasma cells. Plasma cells were found in the vessels in the neighborhood of the foci.

The second case was one of hæmatogenous abscesses of the kidney following carbuncle of the neck and thrombosis of the lateral sinuses. The kidneys contained numbers of small abscesses, which were most numerous in the boundary zone of the pyramids. Cultures showed a general infection with staphylococcus aureus. Sections showed typical metastatic abscesses with masses of large micrococci in the centre, and around these a mass of pus cells with much nuclear detritus. In the tissue on the outside of the abscesses there were numbers of large epithelioid cells which often contain micrococci and other cells inclosed in them. Outside of these there was extensive infiltration, with plasma cells. Plasma cells were found in the vessels.

The third case showed both processes, a general interstitial nephritis and small abscesses with surrounding infiltration with plasma cells. This was from a case of scarlet fever complicated with extensive abscess-formation beneath the scalp. Cultures showed a general staphylococcus infection.

In all three cases the spleen was enlarged, and on section showed large numbers of plasma cells in the tissue.

SUMMARY.

Acute interstitial nephritis is found in the infectious diseases of children, particularly in diphtheria and scarlet fever, but may be met with in other infectious diseases.

The disease is characterized by general and focal infiltration of the interstitial tissue of the kidney with cells which correspond to those which Unna has described under the name of plasma cells. The focal character of the infiltration is marked; even in the cases in which all parts of the kidney show some interstitial cellular infiltration the cells are most abundant in certain foci. These foci are found in three places: in the boundary zone of the pyramids, in the subcapsular region of the cortex, and around the glomeruli. A considerable number of cases is found in which the blood-vessels of the boundary zone of the pyramids contain numbers of lymphoid and plasma cells without any infiltration of the interstitial tissue. The new cells in the interstitial tissue are due to emigration from the blood-vessels and multiplication by mitotic division of the cells which have emigrated. The cells can emigrate as plasma cells or as lymphoid cells, and the latter may change into plasma cells in the tissues. In the normal individual, plasma cells may be formed in the mucous membrane of the intestine, where they practically form the entire tissue between the epithelium and the muscularis mucosa, and to a limited extent in the spleen. In diphtheria, in scarlet fever, and probably in a number of infectious diseases, plasma cells are formed in great numbers in the spleen and bone-marrow, and to some extent in the lymphatic glands. In the spleen they are formed from the cells of the Malpighian bodies, which are often principally composed of them, and to some extent from the cells in the pulp. They are formed from the lymphoid cells.

No adequate explanation is found for the focal character of the lesions in the kidneys. There is some ground for believing that the physical conditions of the circulation may have something to do with their accumulation in the vessels in certain places. It is also possible that in the interstitial foci there may be soluble substances which exert a positive chemotaxis for them. Such substances may be found in the urine, which may exert its influence on the interstitial tissue in different places. The explanation of the foci cannot be found in primary focal degeneration of the epithelium. Epithelial degeneration in these cases is always present, but it is diffuse. In foci where it is more intense and due to the interstitial changes, polynuclear leucocytes are found in the tissue, in the degenerated epithelium and in the tubules. Polynuclear leucocytes and not plasma cells are attracted by degenerated tissue. The foci are not due in these cases to the local action of bacteria.

In a number of the cases in which interstitial nephritis was found the kidneys were shown to be sterile both by cultures and by microscopical examination. In cases where bacteria were present they were found only in small numbers in cultures and not on microscopical examination, and their connection with the foci could not be demonstrated.

In three cases plasma cells were found in the interstitial tissue in definite bacterial diseases of the kidney. In these cases they were not found in connection with the lesions produced directly by the bacteria, but in the periphery of the purulent foci.

DESCRIPTION OF PLATES XXXVII AND XXXVIII.

PLATE XXXVII.

Fig. 1. Circumscribed focus of interstitial nephritis just beneath cortex. The large vein to the right of the specimen is a branch of the stellate vein. Zeiss AA, No. 2 ocular. All the other figures, with the exception of Fig. 3, are drawn with 1/12 Zeiss, ocular 2. All are drawn by camera.

Fig. 2. Plasma cells in interstitial tissue between tubules; the epithelium of the middle tubule is degenerated, the tubule contains polymorphonuclear leucocytes.

PLATE XXXVIII.

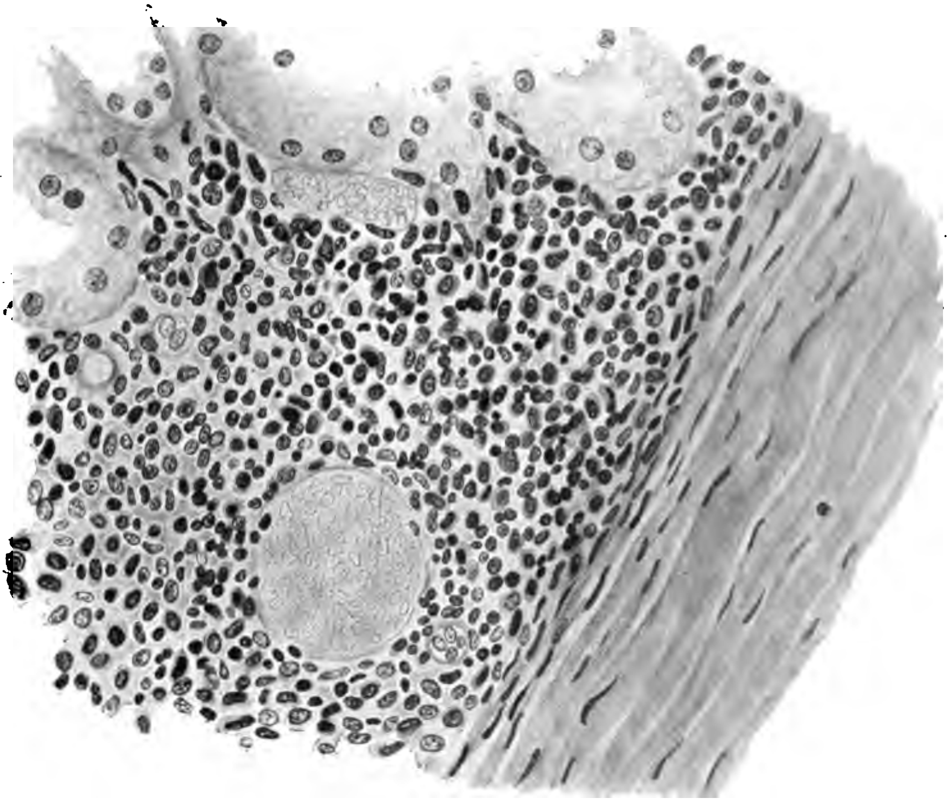
Fig. 3. Section of kidney magnified five times to show the granular appearance of the section, due to interstitial foci.

Figs. 4, 5, 6, and 7. Vessels in boundary zone, containing plasma cells. In 5 and 7 nuclear figures are seen.

Fig. 8. Vessel in boundary zone, containing two degenerated plasma cells.

Fig. 9. Section of vessel of glomerulus, showing plasma cells in vessel.

Fig. 10. Emigration of plasma cell from small vein in boundary line.



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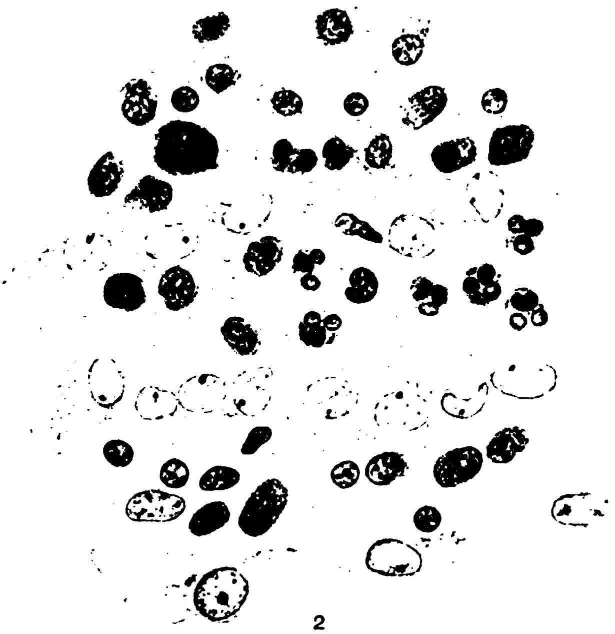

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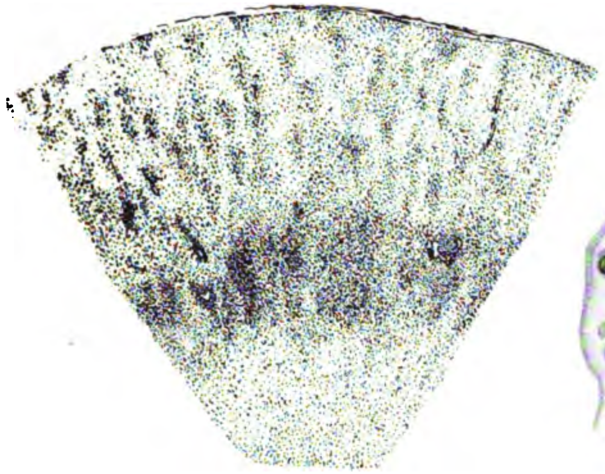

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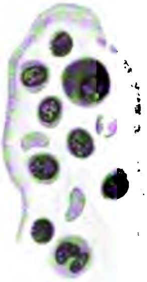

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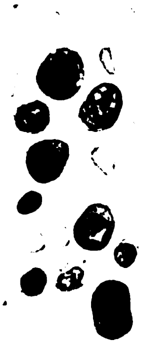

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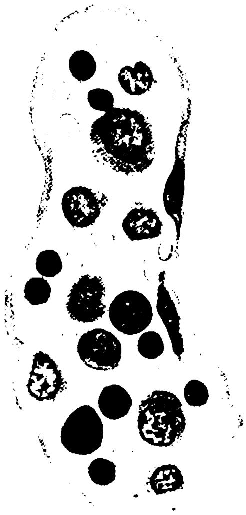

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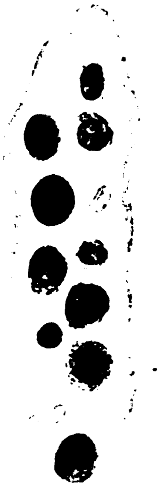

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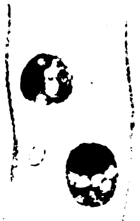

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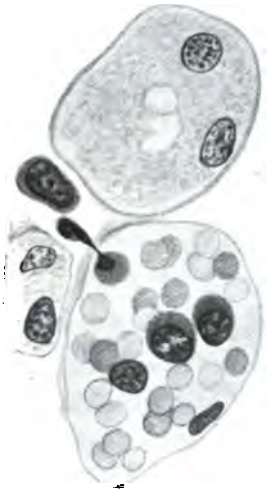

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A CASE OF MYCETOMA (MADURA FOOT).

BY JAMES H. WRIGHT.

(From the Laboratory of the Massachusetts General Hospital.)

PLATES XXXIX AND XL.

Mycetoma is a chronic inflammatory process, most commonly affecting the tissues of the foot, in which suppurative nodular swellings, sinus formation, and enlargement and distortion of the parts are prominent features. The bones may become involved and undergo a rarefying osteitis.

The affection is characterized by the presence in the diseased tissue and in the discharge from sinuses, of peculiar granules, usually not more than one millimetre in diameter, but sometimes larger. These granules in certain cases are of a black color, of irregular shape, and are hard and rather brittle. In general they resemble grains of gunpowder. In other cases the granules are whitish, greyish or yellowish in color and are of a soft or cheesy consistency. They have been compared to fish-roe in appearance. A very few cases are also recorded in which the granules were of a red color. There is no good evidence to show that more than one kind of granules are ever present in any one case of the disease.

On account of the fact that at least two very different kinds of granules are found associated with the lesions, two varieties or forms of the disease are recognized: the "melanoid" or black variety, in which the granules are black, and the "ochroid" or pale variety, in which the granules are white to yellow in color. A. Vandyke Carter, who was the first to show that mycetoma was a special pathological process and to name the disease itself and its varieties, considered the granules to be the cause of the affection and regarded them as representing stages in the life history of one and the same fungus. Carter claimed that he had cultivated this fungus and that it grew as a pink mould. This pink mould was studied by H. J. Berkeley and named by him "Chionyphe Carteri." The connection between this pink mould and the granules was not clearly demonstrated, owing to the conditions under which the cultivation experiments were made, so that the pink mould has not been generally believed to represent the infecting organism in the process. There can be little doubt, however, that Carter saw and recognized in the black granules the same fungus elements which have been described by later observers. This is quite apparent from an examination of the plates in his monograph,* published in 1874.

It is now reasonably certain, however, that the organism of the black granules is of an entirely different character from the organism or organisms making up the pale or yellowish granules. Therefore in summarizing the results of various observers who have studied the nature of these granules, observations on the black granules will be considered separately from those upon the pale or yellow granules.

The disease is endemic in India and has been observed in Africa, Italy and other tropical or sub-tropical countries. In America it is of very rare occurrence, only three undoubted cases, in all, having been reported up to the present time. These are the cases of Adami and Kirkpatrick,† of Hyde, Senn and Bishop,‡ and of Pope and Lamb.§ These were all of the ochroid variety of the disease. In a case reported by Kemper and Jameson,|| the diagnosis is open to serious question, so that it is not included in the list.

Observations on the granules of the melanoid variety. Bristowe¶ studied the black granules in a single case. He found them to consist "of densely aggregated ramifying tubules" embedded in a dark colored matrix. They frequently showed dilatation into round or ovoid cells. These structures he considered to be elements of a fungus identical with

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* A. Vandyke Carter. On mycetoma or the fungus disease of India. London, 1874.

† *Trans. of the Assoc. of Amer. Physicians*, 1895; *Montreal Medical Journal*, January, 1896.

‡ *Journal of Cutaneous and Genito-Urinary Diseases*, xiv (1896), 1.

§ *New York Medical Journal*, September 19, 1896.

|| *American Practitioner* (Louisville), xiv (1876), 129.

¶ *Trans. Path. Soc.*, London, 1871.

that described by Carter. No cultivation experiments were made. Bristowe's description and drawings of the microscopical appearance of the granules are very satisfactory. It is quite evident that he had before him the same organism as that seen by Carter in the black granules.

Hogg * also examined a case of the melanoid variety, finding also fungoid structures in the granules, which, to judge from his description, were evidently the same as those described by Carter and by Bristowe.

In 1888 Bassini † described a case of the black variety which occurred in Italy. The granules were found to be made up of a mycelium of filaments imbedded in a dark brown matrix. These filaments were septate, 4 to 6 millimetres in diameter, and showed irregular swellings. They had a general radiate arrangement in the granule. Besides the granules Bassini noted the presence of strings or cords made up of the same fungus filaments imbedded in a brown matrix. No spore-bearing organs were found and no cultivation experiments were made. The mycelium was regarded as representing " an imperfect vegetative form of a fungus which, in a state of perfect evolution, would be furnished with a true reproductive apparatus." Leucocytes and young blood-vessels were noted in the tissues about the granules.

Kanthack ‡ studied three cases of the melanoid variety in microscopical sections. In all of these cases he found that the black granules seemed to consist, in some instances, of a structureless, olive brown, glassy or finely granular material, in which were imbedded hollow filaments of varying calibre, having a well-marked radial arrangement. Kanthack regarded these appearances as representing degenerative changes, and sought to show that the granules were made up of an organism closely related to actinomyces, as he had found in the case of the pale granules. It is manifest from his description, however, that he saw the same fungus mycelium in the black granules which had been noted by previous observers.

The latest and most satisfactory paper on the structure and nature of the black granules is that of Boyce and Surveyor.§ They studied seven cases of the melanoid form and found in all of them that " the black particles represent a curious metamorphosis of a large, branching, septate fungus." This fungus was imbedded in or surrounded by a brown pigment substance which was readily removed by " Eau de Javelle." The

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* *Trans. Path. Soc.*, London, 1872.

† *Archiv. per le scienze mediche*, 1888, xii (1888), 309.

‡ *Journal of Path. and Bact.*, i (1893), 140.

§ *Trans. of the Royal Society of London*, 1894; *Proc. Royal Soc.*, London, 1893.

hyphæ showed great variations in the size and shape of their segments. They were branched and often disposed in a radiate manner at the periphery. No organs of fructification were observed. The fungus was not cultivated.

Observations on the ochroid variety. Carter's view that the pale particles represented a stage in the evolution of the same fungus as that making up the black granules has already been mentioned.

In 1870 Moxon and Hogg * examined a case of this variety. They found that the granules showed in some instances a texture of fine fibrils, but they were unable to come to any conclusion as to their nature.

Kanthack † examined histologically twelve specimens of the ochroid variety and states that "the parasite of the ochroid variety agrees morphologically and structurally with the typical and perfect ray fungus, and there can be no reasonable doubt that both belong to the same botanical group."

Vincent ‡ found that the granules in a case of the pale form of the disease were made up of a streptothrix, which grew upon potato with a marked red color. In a later paper published with Gémy,§ Vincent reports isolating in cultures the same streptothrix from a second case.

Hewlett ‖ examined two cases of the ochroid variety and states that the granules were very like colonies of actinomyces in structure. He considered that the process was actinomycosis.

Boyce and Surveyor ¶ studied eighteen cases, and conclude that "the white particles consist largely of caseous material and of the remains of a lowly organized fungus, presenting in very many instances some of the characteristics of the fungus of actinomycosis." This organism they regarded as entirely distinct from that found in the seven specimens of the melanoid variety which they examined.

Boyce ** also reports having cultivated a streptothrix from a case of the ochroid variety. This streptothrix differed in its cultural peculiarities from other similar organisms.

Adami and Kirkpatrick †† conclude, from an examination of the case of the ochroid variety observed in Canada, that the granules "were identical in general appearance with actinomyces, forming lobate, reniform masses with central dense mycelium and a radiate arrangement of filaments or clubs around the periphery. These clubs, however, were larger than those of actinomyces."

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* *Trans. Path. Soc.*, London, 1870.

† Loc. cit.

‡ *Annales de l'Inst. Pasteur*, viii (1894), 129.

§ *Annales de dermatologie et syphilographie*, Paris, 3me sér., t. vii, Nov., 1896.

‖ *Trans. Path. Soc.*, London, 1893.

¶ Loc. cit.

** *Hygienische Rundschau*, iv (1894), 529.

†† Loc. cit.

Bishop,* in the paper published with Hyde and Senn, gives the results of his microscopical examination of a case occurring in Chicago. The granules by direct examination were made up of a dense granular central portion and a more transparent peripheral portion in which an obscure radial striation could be observed. No clubs were observed at the periphery. In sections of the tissues the granules consisted of more or less rounded masses, often having a scalloped border and occurring singly and in groups. The central portions stained faintly with hæmatoxylin. Under a high magnifying power delicate filamentous threads, having more or less of a radial arrangement, were to be made out in the masses, and, at the margin of some, delicate radiating lines. There was much to suggest actinomycosis. The tissue changes consisted essentially in the formation of granulation tissue in which giant cells were present.

From a consideration of the results of this list of observers it is quite evident that the disease known as mycetoma is due to at least two different organisms, or in other words, that the black granules of the melanoid form are composed of an organism entirely different from the organism or organisms making up the pale or ochroid granules.

There can be little doubt that the melanoid granules are composed essentially of a fungus or a hyphomycete, and that probably one and the same fungus has been seen by all observers.

The chief thing lacking in these studies of the melanoid granules has been the growing of this fungus. This cultivation has been accomplished in the case reported below.

As to the nature of the granules in the ochroid form it is difficult to form an opinion in some of the cases. It seems clear that, in most of them at least, the granules are composed of organisms of the streptothrix group, to which actinomyces is now generally thought to belong, and not to be the more highly organized hyphomycetes. That some of the cases of the ochroid variety are nothing more or less than actinomycosis seems not unlikely.

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* Loc. cit.

On account of the rarity of this disease in America, and because of the interest attaching to the parasite, the following case is reported:

Clinical History. The patient, an Italian woman, aged twenty-six, was admitted to the service of Dr. H. H. A. Beach, in the Massachusetts General Hospital, December 29, 1897. She was first seen in the Out-Patient Department by Dr. C. A. Porter, who recognized the condition as probably madura foot. The tissues of the left foot in the region of the base of the second and third toes on the plantar aspect presented a swollen appearance with desquamation of the epithelium. In the skin over this swollen area there was a small sinus, from which exuded on pressure a dirty, greyish fluid, containing some black, hard, irregular granules like grains of gunpowder. On the dorsum of the second toe, near its tarso-metatarsal joint, there was a small ulcer.

The disease was first noticed in July, 1897. The patient had lived in America several years. A piece of tissue was excised from the second toe for microscopical examination, and from the results of this, as well as from the appearance and character of the black granules associated with the condition, the diagnosis of mycetoma, or madura foot, of the melanoid variety was made. Amputation of the four toes, together with a part of the four corresponding metatarsal bones, was performed by Dr. Beach.

The dissection of the amputated part showed the following conditions: In the soft tissues of the plantar side of the foot near the tarso-metatarsal articulations and immediately beneath the skin was a pigeon's-egg sized, ovoid tumor nodule, sharply defined from the surrounding tissue (Plate XXXIX, Fig. 1). This nodule on section consisted of a soft, in places gelatinous, myxomatous-like tissue, traversed by an indefinite reticulum, which divided it into indefinite small areas; in these areas were imbedded black, irregular granules less than a millimetre in diameter, resembling grains of gunpowder. These granules occurred singly and in groups. In one or two places the tissue of the nodule was opaque and yellowish, as from necrosis. Two other similar nodules of small size, containing black granules, were also present. One was situated in the soft tissue of the dorsum of the foot near the base of the second and third toes; the other in the soft tissue of the first phalanx of the second toe. The larger of these nodules was about the size of a pea. The bones were not involved.

The structure of the black granules. Macroscopically the granules are of an irregular or mulberry shape, of a black or dark brown color, and usually less than one millimetre in diameter. They are sometimes bound together by shreds of tissue or of exudate. They are hard, rather brittle, and are not readily broken up under a cover-glass.

Microscopically they present the appearance of irregular, lobate masses of a dark brown or black opaque substance. Upon being broken up and examined with high magnifying power, little more than indefinite, brown, somewhat refringent masses can be made out. Occasionally structures suggestive of fungus may be seen, but there is little else to indicate a vegetable nature. They are very resistent to most reagents, but by soaking them for a few minutes, with or without heating, in the ordinary hospital solution of sodium hypochlorite, they lose their brown or black color and become more or less completely white and soft, according to the duration of the action of the reagent. A strong solution of potassium hydroxide also softens them to a certain extent, but does not bleach them so quickly.

Granules from the hardened specimen, bleached and softened as above outlined, when crushed and dissociated under a cover-glass, are found to consist of a mass of fungus structures together with more or less brown pigmented imbedding, or investing material, as described and figured by Boyce and Surveyor,* and others.

Under a high magnifying power (Plate XL, Fig. 7) the greater part of the mass of the granules is seen to consist of ovoid or rounded, translucent bodies of varying sizes, closely packed together, having homogeneous interiors or containing a few refractive granules. Among these bodies typical septate, branching hyphæ may be seen, sometimes showing dilatation or varicosities of their segments. The ovoid or rounded bodies seem to be dilated or degenerated hyphal segments, for not only may they be seen in continuity with the hyphæ, but transitions between them and the hyphæ with swollen segments may be made out.

The periphery of the granules has the appearance of being made

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* Loc. cit.

up of closely set, radiating hyphæ, usually showing more or less swelling or degenerative changes in their segments.

No undoubted spore-bearing organs were observed, but occasionally a large spherical, thick walled body containing smaller rounded or ovoid or elongated refringent bodies was observed. These were regarded as representing some peculiar mode of growth or an "involution," rather than spores and spore-bearing organs.

In specimens treated with caustic potash solution, when the removal of the interstitial pigmentary substance is not complete, branched hyphal tubes may be made out imbedded in a brown refringent substance.

The test for hæmin in the black granules was negative.

In sections of the tissues, under the microscope, the granules are seen as rounded, lobate, reniform or irregular bodies of a more or less reticular structure in general (Plate XXXIX, Figs. 2 and 6, Plate XL, Fig. 8). They are deeply stained by the basic aniline dyes, but are not stained by hæmatoxylin.

In sections stained with the latter they appear to be chiefly composed of a brown-colored, somewhat hyaline substance, arranged throughout the main portion of the granules in the form of a reticulum and in indefinite or rounded clumps. This substance at the margin may merge into a narrow zone of yellow, very hyaline refractive material, in which more or less of a radial striation may sometimes be apparent.

The results of the study of the granules in sections stained in various ways may be summarized as follows:

The granules, as a rule, are made up of a central portion and a cortical portion or rind, without any definite separation between them. In the central portion are usually branching trabeculæ and rounded masses, composed of a hyaline substance, in the midst of which are numerous structures like fungus elements, cut in various planes. In some instances the central portion is made up mainly of an amorphous or indefinite material with only a few fungus hyphæ. In general the central portion is less dense in structure than the cortical portion or rind. The latter in most cases consists of a hyaline material, continuous with the hyaline trabeculae of the central portion, in which fungus elements are imbedded, sometimes arranged more or less in a radiate manner, sometimes not. The margin is irregularly scalloped or dentated. In some cases the cortical portion seems to be made up of more or less atypical hyphæ and elongated processes of hyaline substance radially disposed. In a very few instances the granules appear in the sections as irregularly shaped masses of homogeneous, hyaline or fine granular material, in which unstained or empty fungus tubules or hyphæ are embedded (Plate XL, Fig. 8).

In brief it may be said that the granules are composed of a mycelium of hyphæ or fungoid elements, more or less degenerated, embedded in or surrounded by a hyaline brown-colored refringent substance, which itself forms more or less of a reticulum.

The Gram stain and the Weigert fibrin stain, or modifications of them, stain the substance of the granules, to a variable extent depending on the amount of the decolorizing agents applied. The marginal portions of the granules hold the stain longest. In only a very few instances could the hyphæ be differentially stained by these methods.

Cultures. About sixty-five of the black granules were planted in various culture-media after having been washed in sterilized bouillon to remove contaminating organisms as much as possible. Nearly all of these granules were taken from freshly cut portions of the nodules of the foot after amputation. From approximately twenty-five of the sixty-five granules, a growth of a hyphomycete was obtained. The original cultures, with one or two exceptions, showed more or less contamination with the staphylococcus albus, which was abundantly present in the diseased tissue. The exceptions were in the cases of one or two grains obtained from the sinuses before the amputation and after peroxide of hydrogen had been used at intervals in the sinuses. This reagent had probably destroyed most or all of the pus cocci adherent to the grains, so that the washing in sterile bouillon sufficed to render it free from living contaminations.

The growth in all cases began from the grain and appeared after about four or five days or later. On solid culture-media it first appeared as a tuft of delicate, whitish filaments springing from the black grain. These in the course of a few days increased in number and length, and in the case of the potato formed a dense, whitish or pale brown felt-work or membrane, having a tendency to spread widely (Plate XL, Fig. 12).

Granules planted in potato infusion, in which the contaminating cocci grew only feebly, and granules obtained free from contamination and planted in bouillon, showed after four or more days a faint greyish halo about their peripheries. In the course of some days this increased in size and was seen to consist of long, delicate, radiating filaments (Plate XL, Fig. 9), which grew in length until they reached the wall of the tube and produced the appearance of a powder puff-ball, with the granule in the centre (Plate XL, Fig. 11). Granules were readily obtained free from bacterial contamination and planted in bouillon for development, by selecting those grains which, after remaining on solid culture-media for several days, were well separated from colonies of cocci. Many of the granules, *i. e.* about forty out of the sixty-five, failed to develop any fungus. This fact bears out the idea that many of the fungus elements in the granules are degenerated, as has been mentioned.

The character of the hyphomycete may be summed up as follows:

Morphology (Plate XL, Figs. 8 and 10). Long branching hyphæ from 3 to 8 μ in diameter. In the younger ones delicate transverse septa are present. In the older forms there may be swellings at these points, and the hyphæ may appear as a string of oval-ended, plump segments. The filaments have a definite wall and in their interior, granules or pale areas may be seen. The branching occurs by the formation of lateral outgrowths or buds. No spore-bearing organs were observed.

Potato. It forms a dense, widely spreading, coherent membrane, or layer, of velvety surface, pale brown in color throughout its central portions, and white at its peripheral portions. A marked feature is the appearance of small spherical globules or droplets of dark, coffee-colored fluid on the surface of this layer. The potato takes on a dark brown color and becomes very moist (Plate XL, Fig. 12).

Bouillon. The growth always proceeds from the planted material, whether grain or fragment of mycelium, in the form of fine radiating filaments, and soon produces a puff-ball appearance (Plate XL, Figs. 9, 11). Eventually the whole area of fluid is filled up with the radiating mycelium and a definite mycelial layer is formed at the surface. The medium acquires a deep coffee-brown color.

Potato Infusion. This was prepared by boiling about 20 grms. of potato in water, filtering and making up to 1000 cc. without neutralizing, as used by Vincent.* In this the growth proceeds as in bouillon, except that no surface layer is formed. A peculiarity, however, noted in old cultures consists in the appearance of numerous black granules, about 1 mm. or less in diameter, in the midst of the mycelium.

These granules consist of closely packed spherical or polyhedral cells, together with some short, thick, segmented hyphæ. The walls of these cells have a black appearance and masses of them are black and opaque under the microscope. They seem to represent masses of interlacing hyphæ whose segments have been much shortened and widened, and otherwise changed.

These black masses were kindly examined by Prof. W. G. Farlow of Harvard University, who considered them to be "sclerotia."

Agar-Agar (plain and glucose). The growth appears as a meshwork of widely spreading filaments, of a greyish color, on the surface of the medium. In old cultures black "sclerotia" are found as in the potato infusion. In stab cultures growth appears only at the surface.

No results were obtained from the inoculation of animals with the original granules or with cultures.

Pathological Histology. The tissue composing the nodules consists essentially of a formation of more or less atypical connective tissue, in various stages of development, in which foci of suppuration are present in association with the granules.

Some of the granules lie in small cavities containing polynuclear leucocytes, loose epithelioid cells and cellular detritus. These cavities may be lined either by a wall of vascular granulation tissue or by masses of epithelioid cells, together with multinucleated giant cells

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* Loc. cit.

(Plate XXXIX, Figs. 2 and 4). Other granules are closely invested by a zone of epithelioid and giant cells, and outside of this there may be an infiltration with lymphoid and plasma cells. The nodule thus formed about the granule resembles very closely a tubercle in structure. This tubercle-like formation is shown in Plate XXXIX, Figs. 5 and 6. The giant cells are often of large size and may have a peripheral arrangement of their nuclei. They are a very prominent element in the lesions.

The primary effect produced by the parasites upon the tissues seems to be the development of nodules of epithelioid cells and of giant cells from the tissues immediately about them. Later, suppurative processes occur in the nodules and abscesses are formed, which in time give rise to the development of granulation and connective tissue in large amount.

The writer is under obligations to Dr. H. H. A. Beach, of the Staff of the Massachusetts General Hospital, and to Prof. W. G. Farlow, of Harvard University, for courtesies shown him in connection with the study of this case.

The photographs which accompany this paper were made by Mr. Louis F. Brown. For valuable instruction and suggestions in their preparation the writer is greatly indebted to Mr. John G. Hubbard, of Brookline, Mass.

DESCRIPTION OF PLATES XXXIX AND XL.

All of the figures are photomicrographs from original specimens.

PLATE XXXIX.

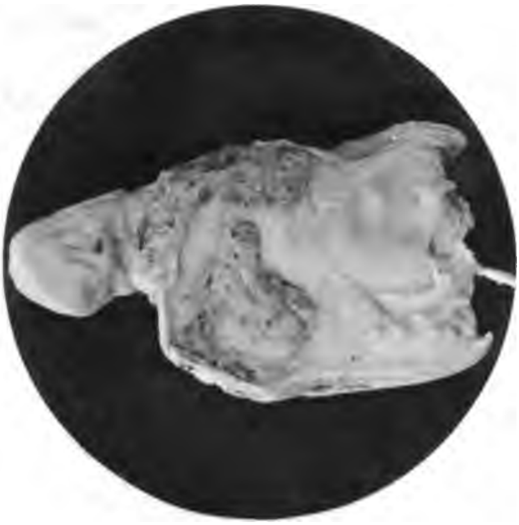
Fig. 1. Portion of amputated part showing the black granules and general character of the lesions as seen with naked eye.

Fig. 2. Section showing black granules and general features of the lesions as they appear under a low magnifying power—Zeiss a_2.

Figs. 3, 4, 5 and 6. Sections showing the granules and surrounding cells. All, with the exception of Fig. 4, taken with Zeiss apochromat. 16 mm. Fig. 4 taken with Zeiss apochromat. 4 mm. Figs. 5 and 6 represent the tubercle-like nodules, with the parasite in the centre, as described in the text.

PLATE XL.

Fig. 7. Fungus elements from a granule after bleaching and softening with sodium hypochlorite solution. A branching, septate hypha is shown. Zeiss apochromat. 4 mm.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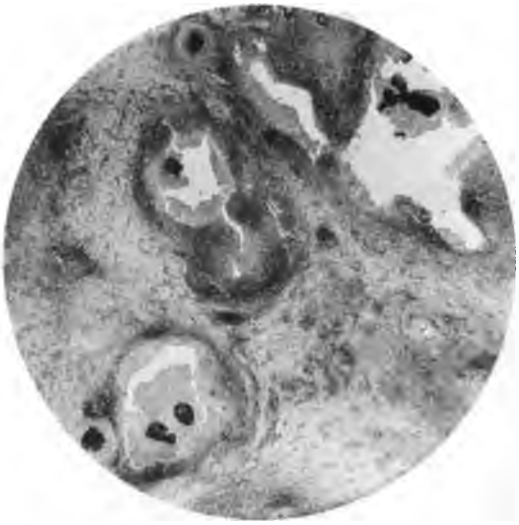
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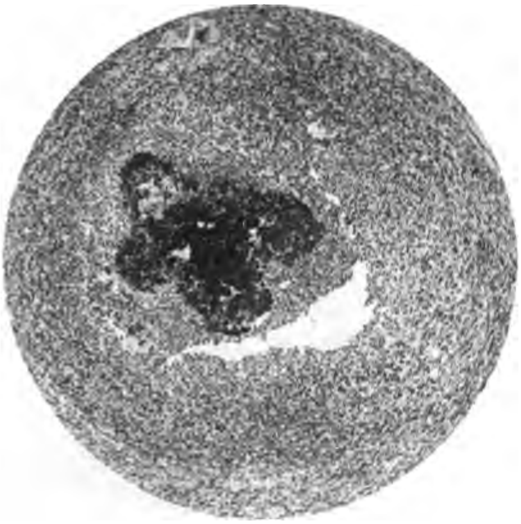
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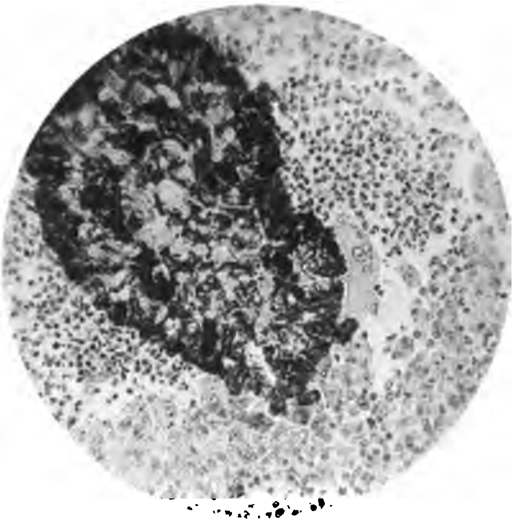
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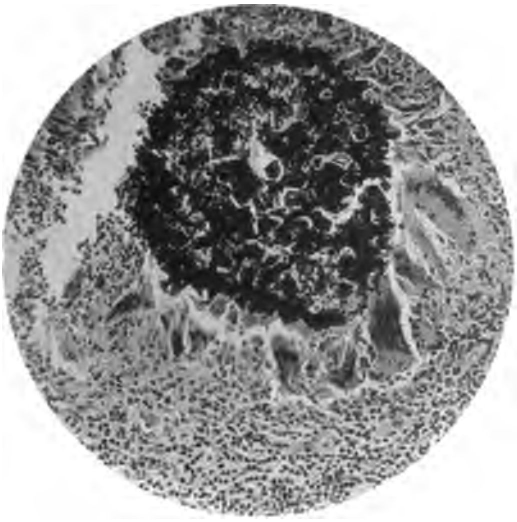
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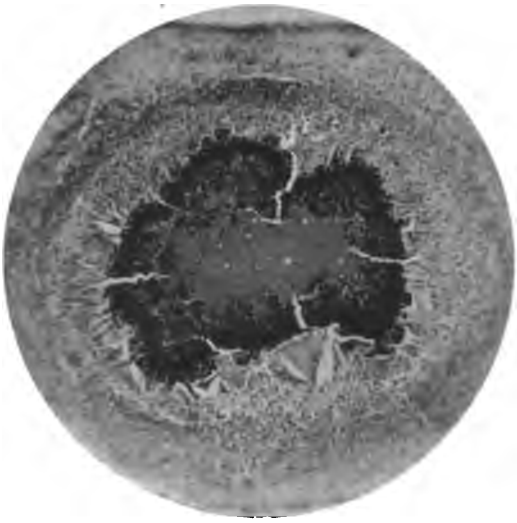
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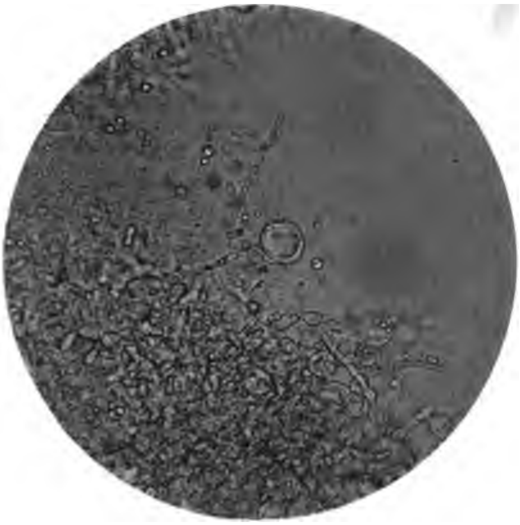

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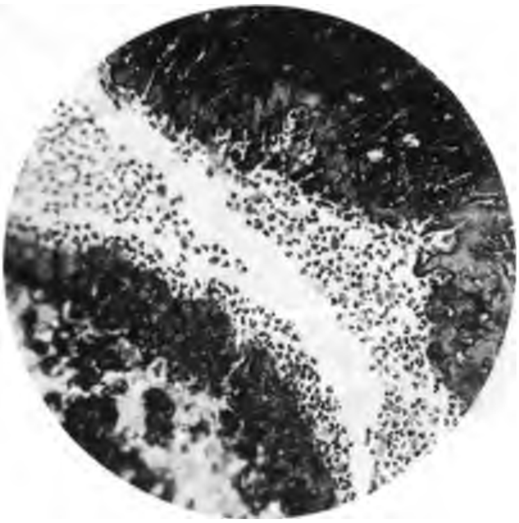

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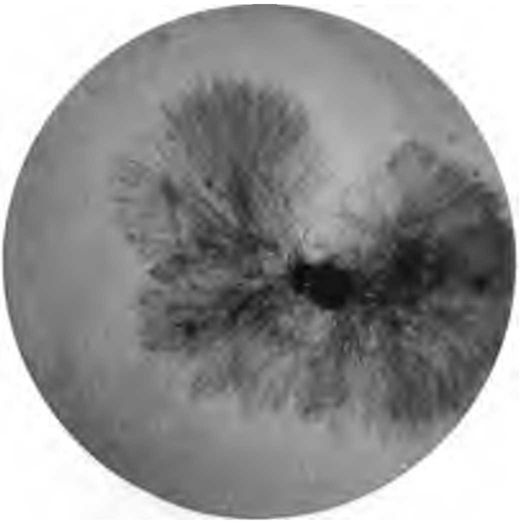

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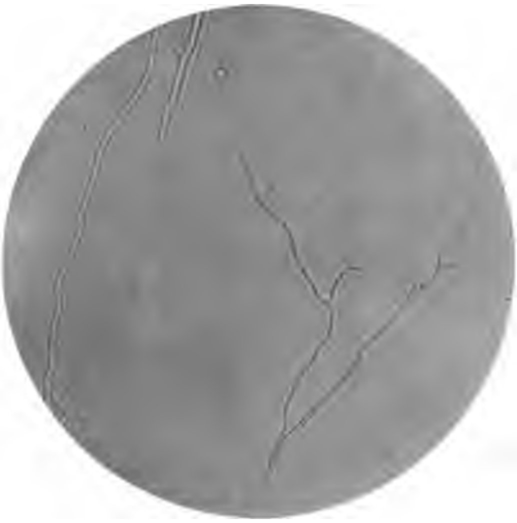

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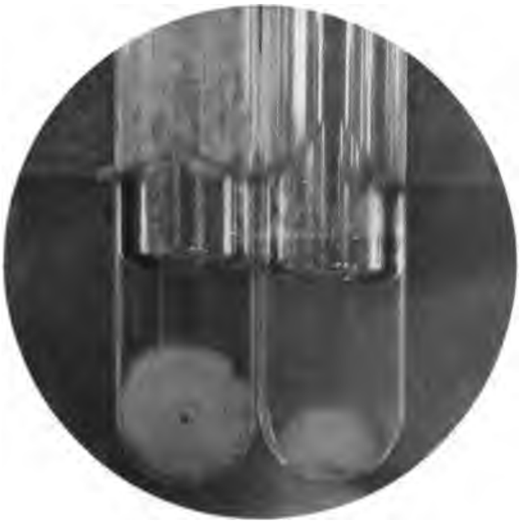

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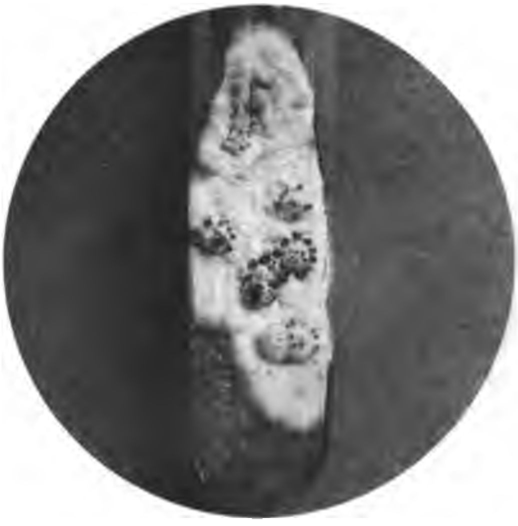

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Fig. 8. Section showing margin of two contiguous granules, highly magnified. Zeiss apochromat. 2 mm.

The small clearer areas represent fungoid elements. The darker substance represents the hyaline imbedding substance.

Fig. 9. Black granule with mycelial growth proceeding from it. Zeiss a_2. Culture in bouillon.

Fig. 10. Showing structure and appearances of the hyphæ of the mycelium obtained from the granules. Zeiss apochromat. 4 mm.

Fig. 11. Two bouillon cultures showing the powder puff-ball appearance. In one the black granule is seen in the centre of the growth.

Fig. 12. Potato culture of the hyphomycete obtained from the granules. The black globules are composed of a dark brown fluid.

PSEUDO-TUBERCULOSIS HOMINIS STREPTOTHRICHA.

BY SIMON FLEXNER, M. D.

(From the Pathological Laboratory of the Johns Hopkins University and Hospital.)

PLATE XLI.

The researches of the past quarter of a century have brought to light many species of micro-organisms whose relations to certain pathological processes have been shown to be of the nature of cause and effect. Among these are included representatives from various groups, bacteria, protozoa, yeasts and moulds. The greatest success thus far achieved has been in the study of the bacteria; indeed, until recently these were regarded as almost the only vegetable micro-organisms capable of causing infectious pathological processes. Our knowledge of the biology of the pathogenic vegetable micro-organisms has more recently been extended in two directions. In the first, certain of the blastomycetes have been proven to be pathogenic for man and animals; in the second, the morphology of the bacteria has been found to depart from the simple formula originally assigned to them. In view of these facts it has been considered necessary to designate a new class for the reception of those vegetable micro-organisms which, while exhibiting affinities with the bacteria, at the same time possess certain features in common with the hyphomycetes. To this class Kruse has given the name *Streptothrices*. Its limits are ill-defined, chiefly for the reason that occasionally one of the common bacterial forms (B. tuberculosis, B. diphtheriæ, B. proteus) will show certain of the characteristics of the group, while for the most part they appear as simple rods.

The streptothrices resemble the moulds in that they develop from spores into cylindrical, dichotomously-branching threads, which alternately grow into colonies the appearances of which suggest a mass of radiating filaments (mycelia). Certain of the threads become fruit-hyphæ, and these break up into chains of round spore-like bodies.

With the bacteria they agree in so far as they lack a doubly-contoured membrane, in not being composed of hyphæ filled with fluid and granular contents and separated from each other by partition walls, and from the fact that they appear as homogeneous threads, resembling the filiform bacteria, which, finally, in older cultures, separate into short bacillary and coccus-like structures (Kruse).*

The researches of Boström † prepared the way for the later studies upon the micro-organism causing actinomycosis and led finally to its classification with the streptothrices under the name of streptothrix actinomyces (Rossi-Doria). Another pathogenic species is represented by streptothrix maduræ (Vincent). These two are the best known pathogenic forms; and an extensive study of their characteristics shows that it is not improbable that they may represent genera rather than species, so that ultimately it may become necessary to distinguish different species or, perhaps, only varieties of each.

The remaining streptothrices described in connection with pathological processes in man are much less well defined. All the pathogenic possibilities of the class have not as yet been discovered. Rosenbach ‡ found a branching micro-organism, capable of growing upon artificial media and of reproducing upon inoculation a similar pathological condition, in an affection known as *erysipeloid* (Erythema exsudativum multiforme). Eppinger § isolated from a brain abscess, which had ruptured into the lateral ventricle and set up meningitis cerebrospinalis, a branching micro-organism which he regarded as a cladothrix (C. asteroides), but which by Kruse is classed with the streptothrices (S. eppingerii). Eppinger injected cultures into guinea-pigs and rabbits and observed that it caused a typical pseudo-tuberculosis. Indeed, the cerebral abscess suggested the disintegration of a large tuberculous focus. The case reported by Garten ‖ is not so clear.

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* Kruse, In Flügge's Die Mikroorganismen, 1896, ii, 48.

† Boström, Untersuchungen über die Actinomykose des Menschen, Ziegler's *Beiträge*, ix, 1890.

‡ Rosenbach, Ueber das Erysipeloid, *Archiv f. klin. Chirurgie*, xxxvi, 346.

§ Eppinger, Ueber eine neue, pathogene Cladothrix und eine durch sie hervorgerufene Pseudotuberculosis (cladothrichica), Ziegler's *Beiträge*, ix, 287.

‖ Garten, Ueber einen beim Menschen chron. Entzündung erregenden, pleomorphen Mikroben. *Zeitschr. f. Chirurgie*, xli, 257.

This author obtained in pure culture a branching micro-organism from abscesses and a fistulous tract, extending from the upper dorsal vertebræ to the sacrum, the ribs and vertebræ showing erosions. While he regards the organism as a new species, its distinction from streptothrix actinomyces is by no means clearly established.

Additional instances of streptothrix infection are reported by Sabrazès and Rivière,* and by Ferré and Faguet.† The first case of Sabrazès and Rivière occurred in a man in whom abscesses were found in the brain, lungs and kidney (softened infarction). The pus from the brain abscess showed single and radiating, branching threads; in the kidney the filaments were much shorter, while in the lungs neither form was discovered. In cultures cocci were found associated with the streptothrix, the latter growing anaerobically only. In a second case these observers isolated from sputa and from a subcutaneous abscess in a man an ærobic streptothrix, pathogenic for small animals. Ferré and Faguet obtained in pure culture from an abscess of the brain in an epileptic, a streptothrix which proved, however, to be non-pathogenic for guinea-pigs and rabbits.

It is now well known that under certain unusual conditions the bacillus tuberculosis is capable of appearing in an irregular branched form.‡ Further observations are, however, necessary before accepting as final the results obtained by Friedrich § and Babes and Levaditi,|| who inoculated cultures of the bacillus tuberculosis into the carotid artery and beneath the dura mater, respectively, in rabbits, and found that the bacilli had not only branched but had also developed into rosettes resembling the "Drusen" of actinomyces.

Another streptothrix pathogenic for man is one upon which a pre-

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* Sabrazès et Rivière, Sur un streptothrix rencontré dans un cas d'abcès du cerveau et d'infarctus suppuré du rein. *La presse médicale*, 1894, Sept. 22.

† Ferré et Faguet, Streptothrix et abcès du cerveau, *Mercredi médical*, 1895, 441.

‡ For the literature on this subject see Craig. The branched form of the Bacillus tuberculosis in sputum. *Journal of Experimental Medicine*, iii (1898), 363,

§ Friedrich, Ueber strahlenpilzähnliche Wuchsformen des Tuberkelbacillus im Thierkörper, *Deutsche med. Wchnschr.*, xxiii, 1897, 653.

|| Babes et Levaditi, Sur la forme actinomycosique du bacille de la tuberculose, *Arch. de méd. exper.*, 1897, 1041.

liminary note appeared in the Johns Hopkins Hospital Bulletin * for June, 1897, and of which the present paper will contain a more complete account.

A consideration of the morphology of the peculiar micro-organism, which was associated with consolidation, necrosis and early cavity formation in the lungs of a human being, and the nature of the pathological process, which in so many ways suggested the caseous pneumonia of true tuberculosis, led to the adoption of the name *Streptothrix pseudo-tuberculosa* for the organism, and *pseudo-tuberculosis hominis streptothricha* as a designation for the pathological condition.

The clinical history of the case offered nothing of especial importance. The patient was a male, colored, aged 70 years, in whom extensive consolidation was made out in both lungs. The symptoms were broadly those characteristic of pulmonary tuberculosis. Sputum was carefully watched for during his stay in the hospital, in Dr. Osler's service, but none was obtained. No microscopical examination could therefore be made.

The autopsy was made 19 hours after death, the body in the meantime having been kept on ice. No evidences of post-mortem decomposition were noticeable. The description of the viscera is limited here to the organs which exhibited pathological alterations.

The *lungs* are voluminous and meet in the middle line anteriorly. They are not bound to the chest wall. *Left.* All of the upper lobe, except the anterior edge, is consolidated more or less perfectly. Where the consolidation is complete, as in the apical portion, the lung presents an opaque appearance, and is grey in color; beginning softening (disintegration with early cavity formation) is going on. These cavities often still contain the products of disintegration, and all appearances of reactive encapsulation are wanting. The lower zone of this lobe is more flaccid and contains scattered areas of consolidation between which the lung tissue is œdematous and swollen, although not completely airless. The lower lobe is congested and œdematous; it contains a number of scattered and partly calcified nodules which on an average are the size of a split pea. The pleura over the hepatized area is covered with a fibrinous exudate. *Right.* The middle lobe is almost completely consolidated. The other lobes contain several small calcified nodules and a number of recent, tolerably circumscribed, caseous areas, averaging in size that of a walnut and surrounded by œdematous, imperfectly consolidated and dark lung substance. The lower lobe contains an almost globular, infiltrated area, 4.5 cm. in diameter, caseous in appearance, the centre of which is undergoing disintegration. The pleura over the fresh foci of consolidation is covered with fibrin and studded with small hæmorrhages.

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* Flexner, Pseudotuberculosis Hominis Streptothricha, a preliminary note, *Johns Hopkins Hospital Bulletin*, vii, 1897, 128.

Peritoneal Cavity. The intestines are moderately distended. The omentum is rolled up and thickened; it occupies a position beneath the transverse colon and extends across the abdominal cavity. The pelvis contains about 15 cc. of fluid of a brownish color and mucilaginous consistence. Between the intestinal loops delicate threads of fibrin exist. In addition smaller and larger nodules, resembling tubercles, usually translucent, are scattered irregularly over all the exposed peritoneal surfaces, and occur more uniformly upon and within the thickened, rolled-up omentum. The liver and spleen on section show similar nodules. The mesenteric glands are not perceptibly enlarged, but upon section minute, opaque tubercles are visible in them.

Histological Examination. The microscopical study of the tissues of the case embraced all the organs. Reference will be made here only to the lungs, the peritoneum and the omentum, in which organs the chief lesions existed.

The pathological process in the lungs consists chiefly of an exudation, which is not, however, of uniform character. For the most part the exudate is cellular, but in certain situations it is composed largely of fibrin.

A stained section of the lung macroscopically presents a variegated appearance due to this heterogeneous exudate. Where this is cellular, a deep nuclear staining is evident; elsewhere the tissue appears pale. The cellular exudate is intra- and inter-alveolar; it is rare, indeed, that the alveolar walls are free from infiltration. The cells composing it are chiefly polymorphonuclear leucocytes, but what is most striking is the extent of fragmentation which these cells have suffered. A small amount of fibrin exists among the cells. Many alveoli contain fibrin almost to the exclusion of leucocytes; this fibrin is dense and compact and encloses cells of the epithelioid type. In certain situations, especially near the somewhat thickened pleura, the fibrin in the alveoli is undergoing rapid organization. The pleura is covered by a fibrinous membrane 3 or 4 times the normal thickness, capped by a thin line of dense fibrin in process of organization.

There is a marked tendency for the tissue showing the cellular infiltration to break down, with formation of cavities showing softened contents. It is found that this softening has taken place only where the infiltration of the alveolar walls has reached a high degree. But the gross examination of the lungs gave no adequate idea of the extent of this process of disintegration, many of the foci being microscopical in size. The disintegrating tissue differs from the general cellular infiltration only in containing more nuclear fragments and irregularly staining detritus. In specimens stained with hæmatoxylin and eosin this irregular material takes on a peculiar, imperfect hæmatoxylin tint, resembling that shown by certain kinds of caseation in tuberculous forms of pneumonia. In the regions in which the exudate is purely or largely fibrinous no such tendency to softening and disintegration is manifest. There is no attempt at limitation or encapsulation of the areas of cavity formation by a growth of demarcating connective tissue.

Circumscribed nodules having the size, form and appearance of miliary tubercles occur in small numbers. They are composed chiefly of epithelioid cells, some of which are disintegrating. Giant cells are not seen.

The bands of interlobular tissue are thickened by a new growth of tissue, and islands of fibroid tissue appear here and there in the lung substance. The blood-vessels are extensively obliterated. This obliteration is caused either by an endarteritis or by thrombosis; sometimes both processes are met with in the same vessel.

Of the structures contained within the abdominal cavity, the intestine and omentum more especially were subjected to histological study. Of these the latter gave the more interesting results. The thickening of the omentum is caused chiefly by a growth of tubercle-like nodules, which cover its surface and also occupy its interior. The nodules are, on an average, larger than miliary tubercles, and still larger masses are formed by the coalescence of several such foci.

In general two kinds of nodules are distinguishable. The larger, probably the more numerous, exceed in size a military tubercle; they are composed of a central mass of disintegrated or disintegrating cells and nuclei. Very irregular nuclear forms are met with, some of these doubtless being emigrated polymorphonuclear leucocytes. Nuclear figures occur now and then among the degenerating cells, but these are taken to be examples of karyorrhexis and not as representing true cell division. Definite fragments of nuclei are common. The centre of such a nodule is occupied often by a transverse section of a small vessel in which white blood-cells can be seen now and then; the lumen is lined by endothelial cells, usually in increased numbers. Beginning at the thin outer wall and often taking in everything except the endothelial cells, a homogeneous material may be noticed, which extends for a variable distance into the tubercles proper, obliterating cell-boundaries as it spreads. With Weigert's fibrin stain this substance takes on a distinctly blue color. The more peripheral parts of the nodules contain more unaltered cells, these presenting the usual appearance of epithelioid cells, although more elongated types (fibroblasts) and irregular leucocytes are also met with. The most external cells are lymphoid, and these, together with more or less polymorphonuclear leucocytes, go over into the surrounding adipose tissue and coalesce with other nodules. Giant cells appear rarely in the less degenerated parts.

In the sections stained with hæmatoxylin and eosin a delicate fibrillated network can be made out among the cells and fragments, which, in specimens stained especially for it, is found to consist of fibrin.

The smaller nodules differ from these in that they are made up of cells which are well preserved and possess large nuclei of the epithelioid type. The cell protoplasm is relatively abundant, and a suggestion of fibrillation is visible in the substance between the cells. This kind of nodule is found in the peritoneal covering of the intestines, where they form an almost uniform layer. Almost no degenerated cells are met with in these formations.

Giant cells are present within the nodules, but they are not abundant there, and they occur without these in the more diffusely infiltrated tissues. Their forms correspond with those found in ordinary tubercle, showing as a rule the peripheral disposition of nuclei and the central hyaline protoplasm. But multinucleated giant cells either with peripheral or centrally massed nuclei occur with especial frequency within the lumina of small blood and perhaps lymph-vessels. It sometimes happens that such a cell occupies the central vessel in one of the first nodules described; but it is more common for them to appear in somewhat larger vessels outside or just at the periphery of the nodules. They are derived from the endothelial lining of the vessels which is met with in the several stages of proliferative activity leading to their formation.

The bacteriological examination. The examination for bacteria consisted in the study of cover-slips from the fresh lungs, the inoculation of glycerine-agar tubes, and the subcutaneous injection into a guinea-pig of a suspension from the consolidated lungs. After hardening the tissues, sections from the lungs, from the peritoneal covering of the intestines and from the omentum were stained for bacteria. Cover-slips from the lungs stained by Gabbett's method showed no micro-organisms which resembled the bacillus tuberculosis in their morphology, but there remained faintly stained in carbol-fuchsin upon the cover-slips numerous examples of a branching organism occurring often in clumps or convoluted masses, among which no ordinary bacillary forms were discovered. Through the use of Weigert's method of staining bacteria the micro-organism was rendered suitable for more exact study.

The cultures from the left pleural cavity and the peritoneum remained sterile. Three separate sets were made from the lungs. In all these, at the end of 24 hours, a vigorous growth of a bacillus identified as belonging to the group of B. coli communis had taken place. The streptothrix did not grow.

The guinea-pig showed no reaction to speak of at the site of inoculation, the adjacent lymph glands could not at any time be felt; the animal, however, lost in weight and died at the end of the 9th week, at the autopsy showing great emaciation. None of the lymphatic glands were found enlarged; there were no lesions resembling tubercles in these and other organs, and cultures upon glycerine-agar, made from several sources, remained sterile. Cover-slips from the serous cavities, blood and viscera were negative for any kind of bacteria.

The further study of the organism stained upon cover-slips prepared from the lungs at the time of the autopsy, by Weigert's method, in which carbol-fuchsin is substituted for gentian-violet, shows it to possess the following characteristics: The micro-organism appears only in streptothrix forms, rarely as single branching filaments, but usually as masses, more or less convoluted, in which the several individuals seem at times to participate. This tendency to form convoluted masses renders the length of the filaments variable; some, however, stretch across the field of the microscope (1/12 objective). The thickness varies also, but to a less degree and in general is about the same as that of B. proteus. The branches are often short and rarely capped at the extremity with a conical swelling (conidia?). That these bodies are not actual conidia is, I think, further proved by their occurrence in the course of the filaments. They doubtless belong to the chromatic particles to be described later. There is protoplasmic continuity between the parent filament and the branch. The contours are slightly irregular, the staining sometimes not quite uniform, and very rarely just before its termination a filament will show 2 or 3 cross partitions suggesting the breaking up into as many short rods. Coccus-like forms were not encountered.

An irregularity in staining is one of the common features of the organism, an appearance, moreover, that depends in part upon the extent of the decolorizing process. The more deeply-stained filaments are a little thicker than those which stain faintly, and the chromatic granules or particles which lie close together in the former may be separated in the latter by widths of unstained protoplasm of greater diameter than the stained particles. In the most pronounced cases the chromatic bodies appear to lie within a non-staining homogeneous membrane (Plate XLI, Fig. 1). The possibility that this difference may depend upon other causes, *e. g.* degeneration, cannot be excluded, but the impression obtained is that it is more or less artificial, and has something to do with the degree of staining, etc.

Sections from several different parts of the lungs were stained first in hæmatoxylin and then in carbol-fuchsin, which was followed by pure aniline oil, for the purpose of differentiation and dehydration. The nuclei appear blue; the micro-organisms red. The latter are very abundant. Their relation to the pathological process is very intimate and somewhat as follows: The only parts of the lung tissue from which the micro-organisms appear to be absent are the areas occupied by the fibrinous exudate. The cellular exudate everywhere shows them, but the streptothrix is most abundant, appearing in the largest masses in the areas of rapid disintegration (Plate XLI, Figs. 2 and 3). The masses of the organism are somewhat larger than are met with in the cover-slips and the branching is more perfect, perhaps because the normal relations are less disturbed in the sections. Rarely, a small fragment of a filament or a separate thread will be present in a mass of pus cells and fragments of cells. The streptothrix appears, but in relatively small clumps, within the tubercle-like nodules; more often indeed a single branching filament is met with in these formations.

The staining of the threads is somewhat more irregular than in the cover-slip preparations; they present a granular, almost beaded appearance, and are thinner than under the former conditions (Plate XLI, Fig. 3).

The omentum especially, of the abdominal organs, was studied for micro-organisms. A large number of sections were treated first with carbol-fuchsin in the thermostat and then carefully decolorized with 1 per cent hydrochloric-acid alcohol. Bacteria were never found in these specimens. Other sections were then stained by the method used for demonstrating the streptothrix in the lungs. Almost every specimen prepared in this way showed bacteria, and often in very considerable numbers. They were contained within the tubercle-like nodules.

The forms of micro-organisms met with in this situation are simpler than those in the lungs. So far as could be discovered from many sections, the individual organisms never branch. They appear as rods of unequal lengths and of a curved or angular form. The staining is irregular, a beaded appearance being very common. The "beading" is quite irregular; more so, I am disposed to think, than is the case with B. tuberculosis. In examples showing the most pronounced irregularity, the organism appears as a nearly colorless filament with uneven deeply-staining red dots placed at different intervals from one another. In length as well as in width the rods rather exceed the average dimensions of the tubercle bacillus. It must, however, be admitted that not a few individuals could not be distinguished by their size alone from this micro-organism.

From the foregoing description there can be, I think, no reasonable doubt that the micro-organism encountered in the lungs differs widely from the known forms of B. tuberculosis. This fact is also supported by the negative animal experiment described. On the other hand, it is equally clear that the pathological process set up by it in the lungs bears no little resemblance to one set of changes at least met with in the same organs in tuberculous pneumonias. We notice in both cases the same tendency to infiltration of the framework of the lungs, degeneration of the inflammatory exudate, and to necrosis and solution of the tissues and exudate. In the particular case under consideration one point of difference is to be seen in the dearth of proliferated and desquamated alveolar epithelial cells within the exudate. But this difference is insufficient to characterize the two processes as essentially distinct. On the other hand, the impression is obtained that the solution of the exudate and framework of the lungs is going on more rapidly, from a greater number of centres, than is the case in ordinary cases of caseous pneumonia.

The analogy of the two processes is strengthened by the finding of tubercle-like nodules in the lungs amid the diffuse exudate. These were, it is true, few in number; but in structure they could not be distinguished from veritable tubercles, and they were proven to have been caused by the streptothrix under consideration, by the demonstration of the organism within them. It will be recalled that sections of the lungs stained for tubercle bacilli by the ordinary method failed to reveal the presence of this micro-organism.

When we come to consider the significance of the tubercles in the peritoneal cavity, it must be admitted that the case in favor of their streptothrix origin is less clear and convincing. But I am inclined to the belief that they owe their presence to the same parasite, which, however, owing to differences in the location and function of the parts, appears under somewhat different morphological forms. In view of the great numbers of micro-organisms contained within these nodules, it seems justifiable to conclude that they would have been demonstrated by the usual stains for tubercle-bacilli, had they been of this nature. But I am unwilling to draw very wide-reaching deductions from this fact alone. The differences in morphology of the organism, when compared with the tubercle bacillus, may again be remembered, without, however, laying too much stress on small distinctions. Perhaps the most convincing argument is to be found in the existence of similar nodules in the lungs, whose etiology seems established. In these formations the organism appears under a simpler morphology than when growing freely within the lung substance.

It would not be profitable, nor would it conduce much to a clearer understanding of the variation in morphology, to dwell upon the influence of environment upon the growth and morphological character of the bacteria; but it should be borne in mind that our knowledge of such variation is constantly increasing and even now includes some of the commoner and more ordinary bacterial forms.

If in the comparison of the lesions in the lungs with those of caseous pneumonia, sufficient proof has been brought of their great similarity to these, more conclusive evidence is at hand of the essential identity of the nodules in the peritoneal cavity with the tubercles caused by the bacillus tuberculosis. It is believed that upon histological grounds alone no distinction could be made.

From these considerations there can be little doubt that the streptothrix described is the cause of the pathological process met with in the lungs and peritoneal cavity, and that in the latter the pathological picture of the disease resembles so nearly tuberculosis in human beings that the two diseases can be separated only by the demonstration of the causative micro-organism in each case. It is, of course, not without the pale of probabilities that at some future time a clinical picture differing from that of true tuberculosis may come to be established for this disease.

If this conception of the pathological changes produced by the streptothrix under consideration be true, the process must be admitted to be a form of pseudo-tuberculosis in man for which the name at the head of this paper is proposed.

The question now arises whether a similar pathogenic streptothrix, producing analogous lesions, has thus far been described. There seems little doubt from the descriptions given of the pathogenic species of this organism in the introductory pages of this paper, that the forms there mentioned differ from the one under consideration. At least no good grounds are at hand for concluding that in any case they are identical or even closely related. Not so, however, with the streptothrix described by Buchholz,* whose publication appeared simultaneously with the preliminary communication concerning the streptothrix pseudo-tuberculosa. It may perhaps be profitable to consider Buchholz's findings more in detail.

The patient was a steel worker, 38 years old, who became suddenly ill 5 weeks before admission to the Institute for Infectious Diseases in Berlin. He entered on November 19, at which time there was absolute dulness over the entire right side of the thorax, with rales and bronchial breathing. The sputum showed numerous streptococci but no tubercle bacilli. The fluid obtained by puncture from the pleural cavity contained streptococci. On November 22, 300 cc. of pus evacuated from the pleural cavity again showed streptococci. Death occurred on December 12th. The autopsy showed the right lung to be united throughout with the costal pleura; the left lung was bound to the diaphragm. The portion of lung corresponding with the adhesion to the diaphragm showed a consolidated focus the size of a hen's egg, which on section appeared to be necrotic. In the pleura covering the right lung were two formations which led directly into two large cavities, themselves communicating, filled with purulent contents and exhibiting ragged and uneven walls. The pus had a fetid odor; the remainder of the right lung, excepting the apex, was firmly consolidated. The rest of the organs showed no abnormality. The anatomical picture suggested an advanced tuberculosis, except that the edges of the cavities were more ragged. Tubercle bacilli could not be demonstrated; streptocci were, however, contained in the pus.

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* Buchholz, Ueber menschenpathogene Streptothrix. Ein Beitrag zur Aetiologie des acuten Lungenzerfalls. *Zeitschr. f. Hygiene u. Infectionskrank.*, xxiv, 1897, 470.

The microscopical examination of sections was negative for tubercle bacilli; the histological picture, too, was regarded as showing differences from that of tuberculosis. The infiltrated parts of the lung presented more the appearance of an acute pneumonic exudate; but in places the interstitial infiltration was so marked as to obscure the structure and the cells were undergoing necrosis (fragmentation, etc.). Stained by Gram's method the firm, infiltrated and necrotic areas showed a rich network of delicately branching threads of about the thickness of tubercle bacilli, which never appeared in the form of long bacilli, but suggested "lines sketched by the trembling hand or the gnarled branches of the oak." Coccus-like and short bacillary forms were not found. The streptococci were contained in the portions of the lung which were consolidated, the alveolar walls being thickened and the alveoli in part atalectic. They were also present in the secretion of inflamed bronchi and in the interstitial lymph-spaces, from which situations the streptothrix was absent. The streptococci are regarded as having been secondary invaders. Cultures were not obtained from the lungs, the plates being overgrown with a variety of proteus. Animal experiments seem not to have been carried out. It is interesting to note that the same difficulty in staining the micro-organism was experienced as recorded in my case, and the most satisfactory preparations were obtained by the use of Gram's method, in which aniline-oil replaces the alcohol.

The resemblance of this case to the one described by me is very striking, but there are also points of marked difference. As regards the latter, the absence of all nodular formations suggesting tubercles and the extensive cavity formation in the right lung stand out in great contrast. On the other hand, it is conceivable that the cavities in Buchholz's case merely represent a later and more advanced stage of the condition present in mine. The description of the micro-organism is strikingly like that given for the streptothrix pseudo-tuberculosa, and in further support of their probable identity, I may mention that Dr. Rabinowitsch, who kindly carried slides of my case to Berlin with her, reports that Dr. Buchholz, after examining the preparations and comparing them with his own, regards them as probably identical.

At the last meeting of the Congress for Internal Medicine, held in Wiesbaden, Scheele and Petruschky* exhibited cultures and microscopical preparations of a pathogenic streptothrix obtained from a woman, aged 56 years, whose history, in brief, is as follows:

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* Scheele and Petruschky, Culturen u. Präparate einer menschen-pathogenen Streptothrix-Art. Verhand. des XV. Cong. f. innere Medicin, 1897, 550.

> The patient gave a family history of tuberculosis, but had herself always been well. In November of the previous year she nursed a sister who died of tuberculosis. In February she suffered with what was supposed to be influenza; in March a cystitis developed, and about the middle of April several small tumors appeared in the skin about the joints (hip, sterno-clavicular), and later in the thigh, gluteal region, etc. The tumors suppurated. In the beginning of May the left upper lobe of the lung showed a fairly intense infiltration. The sputum was examined, but no tubercle bacilli could be found; on the other hand, masses of a "fungus" suggestive of penicillium were made out and a similar organism was demonstrated in the pus from the tumors. The patient died on June 6th. The protocol is not given in this preliminary report. The micro-organism is, however, described as having been obtained in pure culture from the pus from the tumors; in the lung it was admixed with the bacillus influenzæ. In both situations it was abundant. The parasite is said to consist of fine, branching threads without showing any evidences of fructification. The complete description is reserved for a later publication.

In this instance it would be merely a matter of conjecture to consider that the lesions in the lungs and the streptothrix are similar or identical with those described by me; indeed, the clinical course of the case is not especially suggestive of similarity. The important consideration for the present is not to attempt a classification of the several pathogenic streptothrix forms now known, but to recognize their importance in human pathology, and more especially that the lesions, which they produce in the lungs, may be suggestive of certain forms of pulmonary tuberculosis both in their clinical course and their anatomical appearance. Moreover, as the parasite is doubtless thrown off with the expectoration, it should be sought in doubtful cases in the sputa; and for this purpose the usual methods of staining for tubercle bacilli are not suitable. I should recommend the use of the modification of the Gram-Weigert stain with which the parasite was demonstrated in smear-preparations and in sections.

I wish to thank Dr. W. M. Gray for the photomicrographs which accompany this paper.

DESCRIPTION OF PLATE XLI.

Fig. 1. Photomicrograph of smear-preparation from lung.

Fig. 2. Photomicrograph from section of the lung. Both show the streptothrix: Fig. 1, free; Fig. 2, in intimate association with the exudation. Photographs by Dr. Gray, of Washington.

Fig. 3. Section of lung stained in hæmatoxylin and carbol-fuchsin. Zeiss 1/12 in homog. immersion objective. The characteristic beading of the streptothrix is well shown.

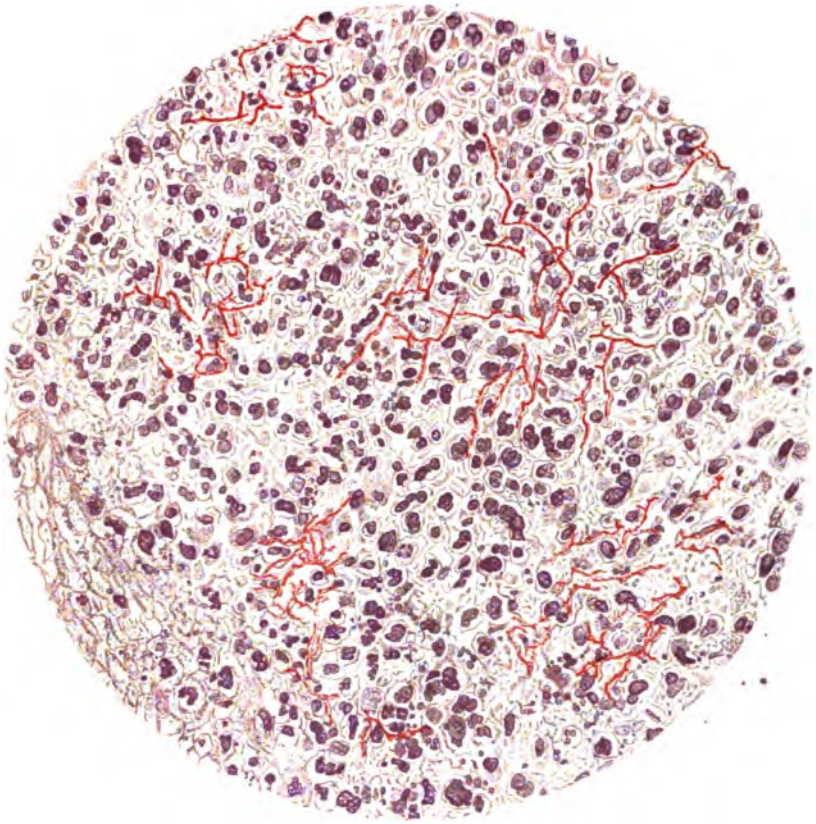

FIG. 3.

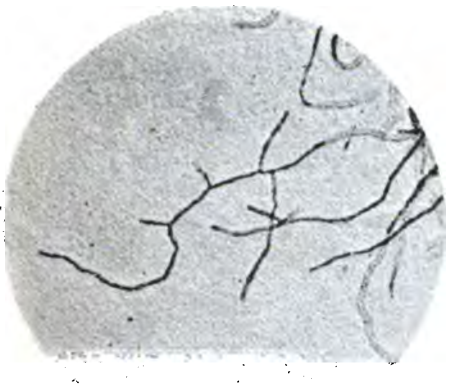

FIG. 1.

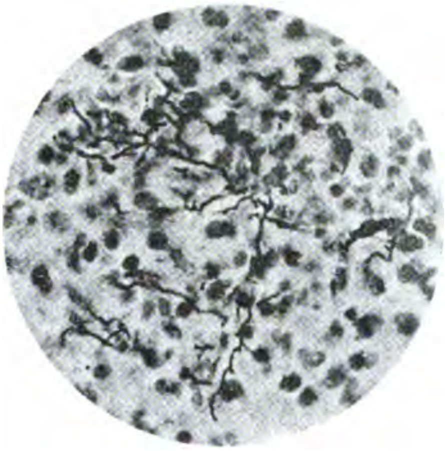

FIG. 2.

A COMPARATIVE STUDY OF BOVINE TUBERCLE BACILLI AND OF HUMAN BACILLI FROM SPUTUM.

BY THEOBALD SMITH, M. D.

(From the Laboratories of Comparative Pathology, Harvard Medical School, and the Massachusetts State Board of Health.)

The absolute identity of tubercle bacilli infecting mammalia has been so generally assumed and the assumption used as a basis for the enactment of sanitary measures having for their object the prevention of any transmission of tubercle bacilli from animals to man, that any one who would attempt to question this identity must be prepared to meet considerable scepticism. Taking a broad biological position, we have every reason to examine into the assumed identity of the bovine and the human bacillus, because both the human and the bovine species are victims of a tuberculosis, presumably transmitted in most cases from one individual to another of the same species, and because the adaptation of a highly parasitic organism to one of these species for centuries may possibly deprive it of much of its power to multiply in the other. Or the reverse may be true. The adaptation of the bovine bacillus to a larger, more vigorous organization may thereby render it more dangerous to man. Assumptions of this sort stimulate inquiry but do not furnish us with positive information. This can be gained only by most tedious investigation.

In 1896 I presented to the Association of American Physicians* a comparative study of a bovine bacillus and a presumably human bacillus which had passed from a tuberculous subject to an animal pet (*Nasua narica*, ' Coati '). The differences which appeared during this work were so clear, both as regards morphological and biological characters, that a further study was indicated. Imbued with the idea that in the minor, though constant, differences of closely related or apparently identical pathogenic bacteria many unexplained phenomena may have their source, and that a comparative study of tubercle bacilli under as nearly the same conditions as can be maintained may lead to results of value to human pathology, I took up the subject anew. A consultation of the literature treating of the character of tubercle bacilli had impressed me with the carelessness shown by most writers subsequent to Koch, concerning the source of the cultures used by them. Papers written on methods of cultivating the presumably human bacillus were really devoted to the avian bacillus. Fischel,* though drawing far-reaching conclusions on the relation of human to avian bacilli, makes no statement concerning his cultures save that they were "mammalian."

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* *Trans. Assoc. Amer. Physicians*, xi (1896), 75.

There being, therefore, no information to be gathered from former publications, the task that presented itself was to study a number of human and bovine tubercle bacilli, and, if possible, tubercle bacilli from other mammalia, side by side, in order to determine whether constant differences, of whatever nature, could be demonstrated. Such differential characters might then be applied in studying different forms of human tuberculosis, especially those cases which are supposed to have been derived from animals, more particularly from cow's milk.

The delay inseparable from the study of this disease has not permitted me to examine more than a limited number of cultures. Including the two described in 1896, 7 sputum and 6 bovine cultures have been isolated and studied; also one animal culture presumably derived from sputum, one culture each from the horse, the cat and the pig.

In Table I are given brief accounts of the subjects from which they were obtained. For the notes on the cases of phthisis I am indebted to a number of physicians who have very generously given their time in tracing the subsequent history.

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* *Fortschr. d. Med.*, x (1892), 908. See also my former article for further references.

TABLE I.

Designation of culture.	Beginning of artificial cultivation.	Source of culture.	HISTORY OF CASE.
Sputum I	July 20, 1896	Peabody, Mass.	Sputum discharged, June 24, 1896, by a young Irish woman. Apex and upper lobe of left lung involved. Slight fever. Sputum contains a considerable number of bacilli. Recovered and worked during summer. Good health in the fall of 1896, with gain in weight. Feb., 1898, in good health, works daily. No cough, expectoration or other symptoms of illness. Over the formerly involved region "the respiratory murmur much diminished down to 3rd rib. Increased vocal resonance, increased fremitus and dullness on percussion." Patient has moved from place to place.
Sputum II	Nov. 21, 1896	New Bedford, Mass.	Sputum containing many tubercle bacilli, discharged Oct., 1896, by a young man confined in prison about one year. Earliest appearance of symptoms not accurately known. Slight cough noticed in Aug., 1896, but no definite physical signs at that time. Grew steadily worse, so that in November he was in bed, weak and emaciated and also suffering with diarrhœa. He died in March, 1897.
Sputum III	Feb. 15, 1897	Norwood, Mass.	Sputum discharged, Jan. 12, 1897, by a man aged 45 years. Duration of disease about six months. Laryngeal tuberculosis chiefly. Difficulty in swallowing and huskiness of voice, which has been gradually increasing. Died Feb., 1897. The physician in charge had drawn off about a litre of serum from thorax 4 or more years ago. Well thereafter until fall of 1896. Apex of lungs slightly involved towards end of disease. Patient has traveled much in this country.
Sputum IV	Feb. 16, 1897	Melrose, Mass.	Sputum containing but relatively few bacilli, discharged Jan. 7, 1897, by a female servant, 22 years old. Physician in charge saw her first Dec., 1896. She complained of hoarseness and cough which began about 6 months ago. Signs of anæmia and cessation of menstrual flow. Placed in hospital, where she remained 8 weeks. At end of April health quite re-established. A subsequent report mentions her visit to Canada after her recovery and her return to Massa-

TABLE I.—*Continued.*

Designation of culture.	Beginning of artificial cultivation.	Source of culture.	HISTORY OF CASE.
			chusetts. She is now (Jan., 1898) at service in another town. She has a troublesome cough and is under the care of a physician.
Sputum V	July 7, 1897	New Hampshire.	Sputum discharged, June, 1897, by a man 25 years old. Had been troubled with a cough for about 2 years, and apices reported involved. Patient feels well in the country, but cannot carry on his trade (printer) in the city. In Jan., 1898, he was reported to be much improved in every way.
Sputum VI	July 14, 1897	Winthrop, Mass.	Sputum discharged, June 16, 1897, by a woman 20 years old. Tubercle bacilli numerous. History not obtainable. Patient died about 6 months later.
Sputum VII	Jan. 18, 1898	Haverhill, Mass.	Sputum containing many bacilli, discharged by an Italian laborer, Dec. 15, 1897. Age of patient 27 years. Returned to Italy in Jan., 1898.
Nasua narica (Coati) human sputum (?)	July 26, 1894	Washington, D. C.	Autopsy, May 12, 1894, revealed primary intestinal infection, as mesenteric glands were farthest advanced in the disease. Omentum studded with tubercles. Extensive embolic tuberculosis of lungs. Household pet of tuberculous man. At Washington Zoological Park, where animal was kept after death of owner, other animals of same species remained well.
Bovine I	Dec. 1, 1894	Virginia.	Bull with advanced generalized disease. Great enlargement of lymph glands of head and thorax. Tuberculosis of the bones. Periodic tympanites due to enlarged mediastinal glands. Killed.
Bovine II	Nov. 28, 1896	Lawrence, Mass.	Cow having multiple necrotic foci in large caudal mediastinal gland. Foci dry cheesy; process evidently old. In one lobe of lung a focus 1¼ inches in diameter with centre softened. In udder small nodules containing actinomyces. Killed.
Bovine III	April 19, 1897	Carlisle (?), Mass.	Cow killed, March 19, 1897, at Brighton (Boston) abattoir. Quite extensive disease of lungs and liver.

TABLE I.—*Continued.*

Designation of culture.	Beginning of artificial cultivation.	Source of culture.	HISTORY OF CASE.
Bovine IV	Apr. 23, 1897	Billerica, Mass.	Cow killed, March 19, 1897, at Brighton abattoir. Disease restricted to one of dorsal mediastinal glands. The gland is moderately enlarged and contains a number of well-defined tubercles, 3 to 4 mm. in diameter, dry cheesy throughout, and easily peeled out of gland tissue. No surrounding infiltration. Evidently a mild stationary case.
Bovine V	Apr. 23, 1897	Carlisle, Mass.	Cow killed, March 19, 1897, at Brighton abattoir. Disease has produced a focus in the lungs, and involves all dorsal mediastinal glands, which are considerably enlarged. One of the portal glands is enlarged, and permeated with a network of necrotic lines.
Bovine VI	Dec. 24, 1897	Newton, Mass.	Caseous matter from a focus in the lungs of a cow 2 to 3 years old. The focus several inches in diameter, probably not more than 3 to 3½ months old, since the cow gave no tuberculin reaction 4 months ago. Dorsal mediastinal gland involved.
Swine I	May 8, 1896	Southboro, Mass.	Lungs and one gland (presumably from mesentery) received at laboratory, March 18, 1896. The herd lived under a cow stable, and the infection was probably bovine in origin. Lungs studded with firm, centrally cheesy or calcified nodules from 1 to 4 mm. in diameter. The lymph gland contained many foci with cheesy centres.
Cat I	Mar. 9, 1897		Case reported by L. Frothingham, V. M. D., in the Journal of the Boston Society of Medical Sciences, March, 1897. Tuberculosis of a mesenteric gland with miliary tuberculosis of lungs, liver and spleen. Tubercle bacilli in capillaries of lungs and liver. Guinea-pig inoculated by Dr. Frothingham, and kindly transferred to me.
Horse I	Feb. 20, 1897		Lungs of a horse from Brighton Rendering Establishment. Guinea-pig inoculated from tubercles by Dr. L. Frothingham. History of animal not known.

It will be noticed that these cultures come from a variety of cases, both mild and severe. Of the patients yielding the sputum bacilli, three are dead, two still sick, one has apparently recovered, and one, a recent case, is now not accessible. Of the bovine cases two were slight, two advanced and two of moderate intensity. Of the other mammals nothing very definite is known.

The bacilli were isolated in all cases by inoculating guinea-pigs with tuberculous tissue or sputum containing bacilli. With sputum the injection of a suspension in bouillon into the abdominal cavity is the most satisfactory. Subcutaneous inoculation may induce a very chronic disease with older (500-gramme) guinea-pigs, which is not so likely to lead to successful cultures because of the scarcity of bacilli in the tuberculosis foci. In nearly all cases the guinea-pigs were chloroformed after three or four weeks, to prevent secondary infections occurring in the final stages of the disease.

Inasmuch as a more general study of tubercle bacilli is desirable, I shall go somewhat into detail concerning the methods I have employed in making cultures. Having had practically nothing but failures until I adopted the procedure to be described, I can recommend it without reserve. There is nothing new about it, excepting in the combination of details. Of 18 attempts to isolate the bacillus, 17 were successful. The one failure was probably due to the slow progress of the inoculation disease in the guinea-pig.

Throughout the work dog's serum was used. The dog was bled under chloroform and the blood drawn from a femoral artery, under aseptic conditions, through sterile tubes directly into sterile flasks. The serum was drawn from the clot with sterile pipettes and either distributed at once into tubes or else stored with 0.25 to 0.3 per cent chloroform added. Discontinuous sterilization was rendered unnecessary. The temperature required to produce a sufficiently firm and yet not too hard and dry serum is for the dog 75° to 76° C. For horse serum it is from 4° to 5° lower. The serum was set in a thermostat into which a large dish of water was always placed to forestall any abstraction of moisture from the serum. About three hours suffice for the coagulation. When serum containing chloroform is to be coagulated, I am in the habit of placing the tubes for an hour or longer in a water-bath at 55°-60° C., or under the receiver of an air-pump, to drive off the antiseptic. This procedure dispenses with all sterilization excepting that going on during the coagulation of the serum. It prevents the gradual formation of membranes of salts, which, remaining on the surface during coagulation, form a film unsuited for bacteria. Tubes of coagulated serum should be kept in a cold closed space where the opportunities for evaporation are slight. They should always be kept inclined.

The ordinary cotton-plugged test tubes I do not use, because of the rapid drying out permitted by them, as well as the opportunities for infection with fungi. Instead, a tube is used which has a ground glass cap fitted over it. This cap contracts into a narrow tube plugged with glass wool. This plug is not disturbed. The tube is cleaned, filled and inoculated by removing the cap. With sufficient opportunity for the interchange of air very little evaporation takes place, and contamination of the culture is a very rare occurrence. In inoculating these tubes, bits of tissue, which include tuberculous foci, especially the most recent, are torn from the organs and transferred to the serum. Very little crushing, if any, is desirable or necessary. I think many failures are due to the often futile attempts to break up firm tubercles. Nor should the bits of tissue be rubbed into the surface, as is sometimes recommended. After a stay of several weeks in the thermostat I usually remove the tubes and stir about the bits of tissue. This frequently is the occasion for a prompt appearance of growth within a week, as it seems to put certain still microscopic colonies in or around the tissue into better condition for further development. The thermostat should be fairly constant, as urged by Koch in his classic monograph, but I look upon moisture as of more importance. If possible, a thermostat should be used which is opened only occasionally. Into this a large dish of water is placed, which keeps the space saturated. Ventilation should be restricted to a minimum. As a consequence, moulds grow luxuriantly and even the gummed labels must be replaced by pieces of stiff manila paper fastened to the tube with a rubber band. By keeping the tubes inclined, no undue amount of condensation water can collect in the bottom, and the upper portion of the serum remains moist. The only precaution to be applied to prevent infection with moulds is to thoroughly flame the joint between tube and cap as well as the plugged end, before opening the tube. When test tubes are employed it is well to dip the lower end of the plug into sterile molten paraffine and to cover the tube with a sterilized paper cap. The white bottle caps of the druggists are very serviceable.

In pursuing this method of obtaining cultures, the question arises: May not attenuated or more saprophytic forms exist in the sputum besides those more pathogenic, and may not the former get into our cultures rather than the latter? This question cannot be answered with entire satisfaction. If we assume, for the sake of illustration, that the original disease was due to perhaps one inhaled bacillus, the progeny of this bacillus may be reinforced by others inhaled which have more saprophytic and less pathogenic tendencies, and which multiply in the cavities and in the sputum to the partial suppression of the original form. If we make cultures directly from sputum this hypothetical danger could not be evaded. The inoculation of the guinea-pig and the three or more weeks elapsing before cultures are made is to a certain degree a safeguard, as it gives the more pathogenic form an opportunity to assert itself by prompt multiplication. I am not inclined to look upon this danger of substitution as a real one. At the same time, it may be best always to choose those successful cultures from the guinea-pig which have come from metastatic rather than local (peritoneal) tubercles. In my work I obtained in most cases cultures simultaneously from 3 or 4 organs, and any differences in these original cultures were not manifest. Only one was used in the comparative studies. Of 7 sputum cultures, 2 were derived from the omentum and 4 from the spleen. Of one the source is not noted. For the bovine cultures no such doubts can be raised, because the foci are closed and not accessible to more saprophytic types.

In the study of the various cultures the conditions were maintained as nearly uniform as possible by using tubes of serum prepared at the same time, and keeping the inoculated cultures in the thermostat in the same trays. For the microscopic examination a little of the surface growth was rubbed between two cover glasses, allowed to dry in the air, and then the whole series to be compared were fixed all together in the hot-air sterilizer at 120° C. for 20 to 30 minutes. For staining, carbol-fuchsin was used cold, and the decolorization effected with a 10 per cent aqueous solution of sulphuric acid acting for from 15 to 30 seconds. Cover-slips were also decolorized with ½ per cent acetic acid, some simply washed in water, others were stained according to Gram-Weigert. In all cases the members of the series studied were treated alike and at the same time, thus eliminating minor, often unavoidable, variations. In all cases the characters to be described refer to dog-serum cultures. These were renewed every 3 to 6 weeks, as many at a time as was possible. The regularity of re-inoculations was now and then interrupted by a beginning infection of the tube with moulds, or a too feeble growth, necessitating earlier transfer to fresh tubes. As a rule the cultures were kept continuously in the thermostat until the next transfer was made.*

The tendency of most species of bacteria to vary slightly from tube to tube, either in size, form, or staining capacity, is also characteristic of tubercle bacilli, so that repeated examination of series of cultures is necessary to obtain what might be called a composite photograph of their most permanent characters or peculiarities. The statements made below as to the morphology of the bacilli under observation are based on the repeated examination of hundreds of slides of which the conditions of growth of the bacilli, the coagulating point of the serum, and the staining manipulations had been carefully noted. The cultivation of tubercle bacilli requires, even under the best circumstances, constant personal attention, and those not prepared to give it had better not attempt it seriously. Success is obtainable along lines other than those suggested by me. Koch succeeded by using beef's serum in ordinary plugged test tubes. He is, however, the only investigator, I believe, who has studied series of cultures of tubercle bacilli.

MORPHOLOGICAL AND BIOLOGICAL CHARACTERS.

With one exception to be noted below (Sputum I), the human bacilli grew from the start much more vigorously than the bovine bacilli. With several the rapidity of growth was surprising. After two weeks these cultures appear as a whitish surface layer of a pearly lustre, of varying thickness. The bovine cultures show merely discrete colonies, or a thin, uniform layer having the appearance of ground glass.† This difference in the vigor of growth has, in general, maintained itself, with the slowly increasing tendency of all cultures to multiply more rapidly.

The size of bovine bacilli in the various cultures was quite constant. They were all quite short, usually about 1 to 1.5 μ long, more rarely 1 to 2 μ. These measurements do not tend to change appreciably with prolonged cultivation. The bacilli are straight, not very regular in outline. Some are broader at one end than at the other, some broader in the middle than at either end, *i. e.* spindle-shaped. Some may be so short as to resemble oval cocci. With the human bacilli the form was not so constant. The earliest cultures of Sput. II, IV, V and VI contained forms from 1 to 2 μ long, hence closely approximating the bovine forms. Others (Sputum III) may be longer from the start. In all, however, there is a tendency, not noticed among bovine cultures, to grow longer under artificial cultivation. Perhaps the greatest contrast in form, which has remained so for three years, is illustrated by the bovine and the Nasua (human) culture described in 1896. In the latter the bacilli are from 2 to 3 times the length of the former.

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* It is always desirable as a precautionary measure to retain 3 or 4 older transfers of any culture in a cool, dark place, so that it may not be lost through some accident or contamination. Now and then culture-media will prove unsatisfactory. In such cases, the earlier tubes must again be resorted to. I have noticed that upon all the serum tubes from one dog, growth was feeble. The liver and the kidneys of this animal showed considerable fatty changes, although the animal had not been ill. Possibly overfeeding may be the cause of the difficulty.

† In some tubes a peculiar coppery discoloration may extend over the entire surface growth. Cultures from these are fertile, however. There is nothing in the microscopic appearance of the bacilli to explain this change. It is not an uncommon feature of cultures of feeble vitality, or of unsatisfactory culture-media.

In the earliest cultures, therefore, morphological differences are not necessarily characteristic, and cannot aid us in attempts at determining the origin of cultures. Cultures containing only small forms have been noted by Metchnikoff and Straus. The latter states that even the avian variety may assume very small dimensions under certain circumstances.

When the serum to be used for cultures is coagulated at a temperature of 71° to 72° C., instead of 75° to 76° C., certain modifications are manifest. All bacilli which have developed on a serum having a firm surface, and which is obtained at the higher temperature, absorb the dye promptly and become brilliantly stained. When a softer (71° C.) serum is employed, whose surface is easily depressed, torn and broken by the platinum needle, the culture, which may have become either more or less vigorous than before, now contains a large number (from 50 to 90 per cent) of bacilli, which stain quite feebly. Uniformly dispersed among them are deeply stained forms. This feeble stain is noticed when bacilli are not treated with decolorizing agents at all, and the appearance of the cover-slip preparation is not materially altered by the acid.*

While both human and bovine bacilli are affected by the softer serum, the former show a much greater degree of change. The bacilli are very feebly stained and show outlines often very indistinct. Further than this, the human bacilli, on the softer serum, tend to become longer and slightly curved. In some cultures this change is very pronounced, in others less so. With the bovine forms there is no elongation, although the bacilli may be less brilliantly stained than on firm serum. Of seven human cultures studied, all have exhibited this tendency to become slender, while none of the bovine bacilli have been seen to change their size to any appreciable degree. Some of the bovine cultures from softer serum did not even show any feebly stained bacilli.

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* Since the completion of the manuscript further studies of this phenomenon have convinced me that at least some of the feeble staining is due to the formation of a capsular substance, which interferes with the penetration of the dye. This substance, which is produced in greatest abundance by the human cultures, is not infrequently detected as an irregular, unstained envelope around a single bacillus or a group. It is not stained by the procedure recommended by Prof. Welch for staining capsules.

Another modification in form which manifests itself upon the firmer serum is the appearance of round, deeply stained bodies, at or near the end of the usually slender bacilli, very rarely in the middle. These bodies, from 1.5 to 2 times the diameter of the rod, take a brilliant stain in carbol-fuchsin, contrasting with the more feebly stained, less compact rod in which they are imbedded. These bodies, which have a striking resemblance to spores, may be present in all the rods of any one preparation, or in a certain proportion only. They have been seen in Nasua, Sputum III, IV and VI. In the bovine cultures bodies of this nature are occasionally recognizable, but owing to the shortness of the bacilli they do not stand out distinctly. Of much more frequent occurrence in these cultures, often the only forms present, are short fusiform or spindle-shaped rods, the central swelling being also deeply stained, and perhaps corresponding to the terminal body of the human forms. These bodies have been seen by Ehrlich, Nocard and Roux, Metchnikoff, Cornil and Babes, Czaplewski and Straus. They have been variously interpreted as spores, and bacilli containing them have been successfully double-stained. Their significance remains unsolved. The most probable explanation is a degeneration or involution form. They may, however, represent some resting stage and correspond to the short, brilliantly stained bodies frequently seen in giant cells, and not recognizable as bacilli. In unstained films, mounted in water, they cannot be distinguished even with a very narrow pencil of light. An increased refrangibility cannot be made out.

Experiments to determine the length of time required at 60° C. to destroy both human and bovine forms have thus far not revealed any differences. The tests are reserved for a special publication.

A study of the relative resistance of the bacilli to drying has not yet been attempted.

Among the sputum cultures is one (No. 1) which grew so feebly on dog's serum that its cultivation was finally given up. The history of the case is briefly given in Table I. Since the comparative tests of the pathogenesis of this culture are very incomplete, I shall give briefly the results of the inoculation of the sputum as evidence that the organism was in reality a tubercle bacillus.

June 27, 1896. Guinea-pig, weight 640 grammes, receives into abdomen 0.5 cc. of bouillon in which some sputum is suspended.

July 20. Weight 457 grammes. Animal rather dull, with coat slightly staring. Very little resistance to handling. Chloroformed.

A few subserous tubercles around point of injection. Omentum represented by a cylindrical mass of neoplastic tissue, about 1 to 2 cm. in diameter, firm, speckled with whitish dots indicative of necrosis. Tubercles on diaphragm and testicles. Liver spotted with small, irregular, not well-defined, yellowish necroses. Spleen slightly enlarged, with Malpighian bodies prominent. Bronchial and sternal glands enlarged, with central necrosis. Lungs free from visible tubercles.

The pathogenic power of this sputum seems to have been equal to that of most sputa examined. One guinea-pig of 672 grammes, inoculated precisely like the preceding, died 46 days after inoculation, with more pronounced lesions of the liver and spleen. A third guinea-pig of 370 grammes, which had received the same quantity subcutaneously, weighed a trifle more on the 49th day (390 grammes) than at the beginning. On this day it was chloroformed and examined. The typical ulcer with surrounding tubercles in the subcutis and a few tubercles in the omentum were seen. The liver was pale and fatty, the spleen enlarged to several times the normal size and uniformly permeated with grayish (one mm.) foci, but without necroses. The lungs were studded with small, partly necrotic tubercles. The bronchial and sternal glands were large, with necrotic centres.

The first culture showed somewhat slender but well-stained bacilli. In tubes subsequently inoculated, the bacilli multiplied almost exclusively in the condensation water, which became milky in appearance. In this the bacilli appeared in roundish clumps among cholesterin crystals. The bacilli were imbedded in a colorless material which formed the cohesive clump. The stained bacilli presented no unusual characters save in one culture (after about six months of total cultivation), in which fully one-half of all the quite slender bacilli appeared as series of transverse, apparently unconnected stained blocks, simulating streptococci. It is unfortunate that more animal tests were not made with this stock. As I was expecting a more vigorous culture from month to month, tests were postponed until conditions should approximate those of the other cultures. These were, however, not realized. The only test upon a rabbit is given in Table III., The cultivation was finally abandoned.

Of the other animal bacilli the cultures from swine were identical with the bovine cultures in morphological and other characters. Those of the horse and the cat were also either identical with the bovine bacilli or they very closely resembled them.

If we undertake to summarize the observations made with microscope and culture tube upon these bacilli, we somewhat hesitatingly formulate the following general statements:

1. Bovine and other animal bacilli (except *Nasua*, which is regarded as coming from man) grew less vigorously for a number of generations than the sputum bacilli. Sputum I is an exception and is probably an atypical form.
2. Bovine bacilli are much less influenced by certain modifications of the culture-medium.
3. Bovine bacilli tend to remain short; human bacilli are either more slender from the start or become so during cultivation.

PATHOGENESIS.

The most important means of demonstrating identity or divergence of characters of pathogenic bacteria is the test upon animals. This test is a purely physiological one, but in the application of experimental results from the domain of bacteriology to medicine, morphological characters must give way to physiological.

That there might be differences among tubercle bacilli from different species of mammals presented itself as a possibility to Koch and is referred to in his monograph. He, however, failed to recognize any differences worth mentioning. There are several reasons for this. In the first place, Koch was at that time endeavoring to prove to the world that the tubercle bacillus is the cause of tuberculosis. Minor details received no attention because of the great and difficult task immediately before him. In the second place, the course of tuberculosis is essentially a function of the number of bacilli introduced. Variations in the dose result in corresponding variations in the length of the disease, in its final termination, and in the extent and distribution of the lesions. This fact Koch himself recognized in discussing the results following the intra-ocular inoculation of varying quantities of culture material in rabbits. He did not, however, attempt to adjust carefully the quantity to be injected or to determine the effect of varying quantities of bacilli upon any of the species used by him. Curiously enough, no one following him seems to have considered it worth while to extend his work by more accurate comparative tests. That such work is likely to be fruitful the following experiments amply demonstrate.

Guinea-pigs. The inoculation of this species with tubercle bacilli from the various cultures brought out few well-defined differences. The great susceptibility of guinea-pigs to tubercle bacilli is responsible for the general acceptance of a common level of pathogenic power for all mammalian tubercle bacilli. This tendency to unify physiological characters because of a more or less uniform action upon very susceptible species has led to frequent failure on the part of experimenters to recognize important variations among species of bacteria, variations probably having much influence upon the course of the disease contracted in the natural way. There are, however, certain differences in the action of bovine and sputum bacilli upon guinea-pigs which enable us to recognize even in these very susceptible animals the much greater virulence of bovine bacilli. It is probable that by a graded dosage of tubercle bacilli more marked differences might be brought out than I am in position to present.

It has already been stated that all the cultures in hand had been obtained by inoculating the tuberculous tissue or the sputum into guinea-pigs and isolating the bacilli from them. To obtain the bovine cultures, a small bit of tissue was placed in a subcutaneous pocket in the flank. For sputum bacilli the sputum was shaken up in sterile bouillon and the dilution injected into the abdominal cavity. In these preliminary experiments differences presented themselves between the two kinds of material. Although the sputum, as a rule, contained very many tubercle bacilli and the bovine tissue very few, yet the subcutaneous injection of sputum led to such a chronic disease, with comparatively slight lesions and a tendency to reparative changes in the liver, that the animals were very unpromising subjects for culture when chloroformed.* Hence the intra-abdominal inoculation in most cases. On the other hand, the disease due to bovine tissue placed in the subcutis never failed to produce extensive lesions, with indications of certain death in 4 to 6 weeks, which was anticipated by chloroform when cultures were to be made. Leaving this preliminary series of inoculations, we will pass to those made with definite doses of tubercle bacilli in suspension.

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* These statements apply only to guinea-pigs weighing 400 grammes and above.

The suspension was made by thoroughly rubbing the growth from blood serum upon the inside of dry sterile test tubes with a heavy spatula-like platinum wire. This procedure results in the breaking up of clumps and the coating of the tube with masses of bacilli. Bouillon was then poured in and the thoroughly stirred suspension allowed to stand for several hours until all coarser particles had subsided. The resulting suspension was diluted, if necessary, until its density was nearly equivalent to that of a bouillon culture of typhoid bacilli 20 to 24 hours old, and nearly as homogeneous. More accurate dosage might be obtainable by weighing the moist growth before suspending it in bouillon; but inasmuch as the weighed quantity may not all be brought into a finely divided state—a matter of importance in the intravenous injection of rabbits—the method adopted is probably less troublesome and equally accurate.

Of this suspension the usual dose, both for subcutaneous and intra-abdominal inoculation, was 0.5 cc. An examination of the dates in the various tables will show that many of the inoculations upon rabbits, guinea-pigs and cattle were made with the same suspension on the same day.

The guinea-pigs employed were all rather large, weighing, with few exceptions, from 400 to 700 grms. They had all been used in diphtheria antitoxin tests one to three months before. The rapid increase in weight was evidence of no disturbance of health at the time of inoculation. It is of importance to bear in mind that the animals were large, because the progress of tuberculosis differs essentially in growing and adult guinea-pigs, the latter manifesting a far greater resistance than the former, a resistance expressed by well-marked reparative or cirrhotic changes in the liver. The differences to be pointed out in the bovine and sputum series may possibly be nearly wiped out by the use of guinea-pigs weighing only 250 to 300 grms.

The disease induced by the intra-abdominal injection was so rapid in its progress for both bovine and sputum cultures that the virulence of both races appears nearly the same.

The shortest period between inoculation and death, when 0.5 cc. of the suspension was injected, is 7 days, the longest 21 days, with one exception, that of Sputum VI, which was 38 days. In other cases the period was prolonged because of smaller doses. For Bovine I (0.2 cc.) it was 33 days; for Nasua (0.3 cc.) 41 days. If we take the largest series in which the injections were made at the same time and in which the conditions were identical—the series comprising cultures of Sput. IV, V, VI and of Bovine III, IV, V—we find the periods elapsing between inoculation and death to be respectively 21, 11, 38 for the sputum and 7, 9 and 10 days for the bovine cultures.

The disease due to the intra-abdominal injection of tubercle bacilli in the quantity used is peculiar in this, that there is always found after death a large quantity of an opalescent fluid in both pleural sacs. This fluid greatly compresses the lungs and is probably the immediate cause of death. When the disease is prolonged beyond 3 weeks, the pleural effusion is absent. A careful histological study of the various organs in this most acute form of the inoculation disease, which will probably furnish the explanation of the effusion, is still to be made. In the abdominal cavity there is, as might be expected, a general inflammation of the peritoneum, associated with some fibrinous exudation and usually a little viscid fluid, when the disease does not last quite ten days. Any prolongation beyond this brings a dense eruption of tubercles into view. The omentum is in all cases represented by a cylindrical mass of fused tubercles.

If we turn to the subcutaneous inoculations, the difference in pathogenic power is more clearly brought out.

In the same series just quoted, of the three sputum guinea-pigs, one died 44 days after inoculation, the others were chloroformed respectively 77 and 100 days after inoculation; the first having lost but little in weight, the second being a trifle heavier, but having extensive pulmonary disease. The bovine guinea-pigs died in 51, 41 and 40 days respectively. The sputum guinea-pig, which succumbed in 44 days, weighed at the start 130 grms. less than the lightest bovine guinea-pig. The lesions were slight, the lymph glands only moderately enlarged, the foci in the lungs not visible to the naked eye. The spleen was not enlarged. The liver was mottled with very many grayish specks. The microscopic examination showed lesions but slightly advanced and reparative changes with proliferation of bile-ducts in progress. In general, the tuberculous lesions in this animal did not warrant so early a death.

The histological examination of the tissues of the infected guinea-pigs was restricted to the lungs and the liver of those inoculated simultaneously under the skin with cultures of Sputum IV, V and VI,* and of Bovine III and V. These organs were chosen because of their peculiar behavior towards the infection. The liver is invaded first, and under favorable conditions cirrhotic changes may begin which in some cases apparently lead to an elimination of the disease, with considerable shrinking of the organ and furrowing of the surface. In the lungs the disease begins later, and if the animal survives the first 6 weeks, may grow steadily until most of the organ is involved.

Pieces of tissue hardened in Zenker's fluid or alcohol were cut embedded in paraffine. The sections, fastened to the slide with Mayer's albumen and water combined, were stained with hæmalum and eosin or picro-acid-fuchsin, or with eosin and methylene-blue. In some cases Weigert's fibrin stain was resorted to. All were stained for tubercle bacilli in carbol-fuchsin, differentiated in 1 per cent acetic or decolorized in 4 per cent sulphuric acid, and stained subsequently in Löffler's methylene-blue. The stain for tubercle bacilli was successful in the tissues hardened in Zenker's fluid. Even in the sections stained according to Gram-Weigert, the bacilli appeared distinctly, but in a peculiar form, often simulating short streptococci with the individual cells rather well separated, or series of short blocks with spaces between them. Here the stain fastens itself only upon certain segments of the bacillus, leaving the connecting elements entirely unstained. The part selected evidently stands in no relation to the bulging, deeply stained, spore-like bodies referred to above as occurring in cultures.

In discussing the histological changes induced by tubercle bacilli in the various animals inoculated, it has been my determination to steer clear of all controversial questions relating to the histogenesis of the tubercle or of the elements composing it, as being foreign to the scope of this etiological study. Only those features will be very briefly referred to which show pronounced variations and which serve to point out differences in the action of the bacilli.

In the liver of the inoculated guinea-pig, the proliferative lesions present in all cases examined consist of aggregations of the characteristic epithelioid cells, among which, here and there, scattering nuclei appear richer in chromatin, and which for convenience are designated lymphoid elements. The lesions, situated chiefly in the interlobular tissue, are surrounded with a wide or narrow zone of proliferating bile-ducts, which appear as single or forked lines or in the form of meshes. There is nothing in the microscopic appearance of the liver tubercles to serve as distinguishing marks, excepting the much more extensive involvement of the liver tissue and the much greater abundance of tubercle bacilli in the bovine cases. In the latter there are added to these lesions those of another character, the simple necrosis of cell territories ushered in by a fading out of the nuclei and a deep stain with eosin. These areas probably represent the yellow cheesy areas so common, and often very large, in young tuberculous guinea-pigs. They are readily distinguishable with the naked eye from the grayish proliferative changes going on in the interlobular tissue. In the sputum cases the very rare areas of this character seen were very small.

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* Spleen in place of liver in this case, the latter having been rejected by mistake.

In the lungs, the lesions are as a rule perivascular and peribronchial. There are no essentially distinctive features here, excepting the very great abundance of tubercle bacilli in the bovine cases. In Bov. III the bacilli are also found in large numbers in cell detritus occupying alveoli and small bronchi. In Sputum VI many alveoli in the periphery of the foci are nearly filled out with multinucleated cells resembling giant cells closely, but their contents are only pigment particles. In Bovine V similar cells are present, though in small numbers. These, as well as other cells in the alveoli, each contain several bacilli.

The action of tubercle bacilli from the pig, the horse and the cat upon guinea-pigs was nearly the same as that of the bovine cultures, and needs no special discussion here.

With this hasty, imperfect sketch we may conclude the notes on the tuberculosis of guinea-pigs induced with cultures, by simply adding that the bovine bacilli studied are more virulent than human bacilli, although, owing to the great susceptibility of this species, the differences are not likely to impress us unless we give much attention to experimental details. These differences may be summed up as consisting in the more rapid death of all guinea-pigs inoculated with bovine bacilli, the greater extent of the lesions and the far greater abundance of tubercle bacilli in them.

In Table II will be found, in part, the basis for these conclusions.

TABLE II.—GUINEA-PIGS.

Culture.	Total age of artificial culture.	Number of transfer.	Date of inoculation.	Age of culture used, in days.	Dose in cc.	Mode of inoculation.	Initial weight in grammes.	Final weight.	Result.
Sputum I	No inoculation of cultures.		(Of sputum suspension) June 27, 96.		0.5	intra-abd.	672	430	Dies Aug. 11–12.
					0.5	sub-cut.	370	390	Chloroformed August 15 (active).
					0.5	intra-abd.	640	457	" July 20 (sick).
" II	3 mos. 3 days.	5th	Feb. 24, 97.	24	0.5	sub-cut.	570	520	Chloroformed April 19.
					0.5	intra-abd.	600		Dies March 6–7 (10–11 days).
	5 " 10 "	8th	May 1, 97.	9	0.5	" "	440	312	" May 13 (13 days).
" III	1 " 19 "	2d	April 3, 97.	18	0.5	" "	525		" April 12–13 (9 days).
	2 " 17 "	4th	May 1, 97.	9	0.5	" "	435	245	" May 17 (17 days).
" IV	2 " 16 "	2d	May 1, 97.	18	0.5	" "	705		" May 15–16 (15 days).
	6 " 10 "	7th	Aug. 26, 97.	9	0.5	sub-cut.	372	275	" Oct. 9.
	6 " 10 "	7th	Aug. 26, 97.	9	0.5	intra-abd.	507		" Sept. 16–17 (21 days).
" V	1 " 19 "	3d	Aug. 26, 97.	9	0.5	sub-cut.	458	402	Chloroformed Dec. 3.
					0.5	intra-abd.	517		Dies Sept. 5–6 (11 days).
" VI	1 " 12 "	3d	Aug. 26, 97.	9	0.5	sub-cut.	420	431	Chloroformed Nov. 11, extensive lung disease.
					0.5	intra-abd.	478	338	Dies Oct. 3 (38 days).
" VII	1 " 10 "	2d	March 1, 98.	16	0.5 *	sub-cut.	323	360	Dies May 14–15.
					0.5 *	intra-abd.	332	222	Dies March 18–19 (18 days).
Bovine I	15 " 19 "	14th	Mar. 20, 96.	16	0.5	sub-cut.	405	?	Chloroformed May 14 (failing).
					0.2	intra-abd.	478		Dies April 22 (33 days.)
" II	2 " 27 "	3d	Feb. 24, 97.	24	0.5	sub-cut.	580	?	" Mar. 24.
					0.5	intra-abd.	645	?	" Mar. 3–4 (7 days).
	5 " 3 "	5th	May 1, 97.	9	0.5	" "	505	?	" May 13 (13 days).
" III	4 " 7 "	5th	Aug. 26, 97.	9	0.5	sub-cut.	533	400	" Oct. 17–18.
					0.5	intra-abd.	624	?	" Sept. 2–3 (7 days).
	9 " 27 "	8th	Feb. 15, 98.	17	0.5	" "	581	468	" Mar. 2 (15 days).
" IV	4 " 3 "	5th	Aug. 26, 97.	9	0.5	sub-cut.	635	400	" Oct. 6–7.
					0.5	intra-abd.	533	?	" Sept. 4–5 (9 days).
" V	4 " 3 "	5th	Aug. 26, 97.	9	0.5	sub-cut.	504	336	" Oct. 5.
					0.5	intra-abd.	490		" Sept. 5–6 (10 days).
" VI	2 " 18 "	3d	Mar. 15, 98.	13	0.5 *	sub-cut.	406		Dies May 2–3.
					0.5 *	intra-abd.	423		Dies April 7 (23 days).
Nasua	19 " 22 "	18th	Mar. 20, 96.	16	0.75	sub-cut.	455	?	" April 26, probably chronic kidney disease.
					0.3	intra-abd.	505	?	" May 1 (41 days).
Swine I	2 " 6 "	14th	July 14, 96.	12	0.5	sub-cut.	525	?	" Aug. 7.
					1.0	intra-abd.	525		" July 25–26.
	11 " 23 "	16th	May 1, 97.	13	0.5	" "	450	300	" May 15 (14 days).
Horse I	5 " 4 "	6th	July 24, 97.	18	0.5	sub-cut.	395		" Aug. 19–20.
					0.75	intra-abd.	415	290	" Aug. 7 (14 days).
Cat I	4 " 15 "	5th	July 24, 97.	18	0.75	sub-cut.	424	290	" Aug. 28–29.
					0.5	intra-abd.	427	?	" Aug. 8 (15 days).

* Dilution one-third to one-fourth of that hitherto used.

Rabbits. The inoculation into the anterior chamber of the eye has been the most widely practised method. This seems to have been followed by variable results, whether due to the number of bacilli injected, the source of the bacilli or the amount of injury inflicted on the eye.

Koch injected tubercle bacilli from various sources into an ear-vein. The bacilli were suspended in blood-serum and the suspension passed through fine gauze to eliminate coarser particles. 4 rabbits were inoculated in this way with bacilli from a monkey, 3 with bacilli from a case of phthisis and 3 with bacilli from cattle. The rabbits all succumbed in from 18 to 31 days, excepting one, which was killed on the 38th day. In another experiment a culture from a case of lupus and from a monkey were tested on rabbits. Death ensued in 2 to 3 weeks. The weight of the rabbits and the density of the suspensions used are not given, so that comparisons are impossible. Moreover, the medium used—liquid serum—may not be entirely indifferent. Straus* mentions the slight effect produced upon rabbits by cultures of human tubercle bacilli.

In the present work, the injection of tubercle bacilli into an ear-vein was used at first tentatively, then, when sharp distinctions between bovine and human cultures were made manifest by this procedure, it was continued.

Rabbits weighing from 1300 to 2200 grms. were employed. The suspension was prepared as already described, and for most of the primary tests 0.5 cc. was injected. By referring to Table III it will be seen that for the primary test of the last pair of cultures, Bovine VI and Sputum VII, the suspensions were ⅓ to ¼ as dense and the dose was doubled. The other cultures of which larger doses were injected are Sputum I, Nasua and Cat I (0.75 cc.), and Swine I (1 cc.). More recent tests indicate, however, that any variation in the dose as slight as these has but little influence in modifying the duration of the disease.

As far as practicable, the tests were made as soon as the purity of the cultures was beyond doubt, in order to reduce to a minimum the factor of artificial attenuation. This seems to have been on the whole inconsiderable.* An effort was also made to test the cultures in groups as far as possible, in order to furnish identical conditions. These groups are Bovine I and Nasua, Sputum II and Bovine II, Sputum III and IV, Sputum I and IV, Horse I and Cat I, Sputum V, VI and Bovine III, IV and V. Sputum VII and Bovine VI may be considered a group, though the inoculations were not made simultaneously, owing to an accident. The ages of the different groups of cultures varied from a minimum of 9 to a maximum of 24 days. The total age of the cultures likewise varied at the date of the first test from 1 month and 10 days to 9 months and 25 days. These differences, which were largely counterbalanced by the group inoculations, cannot be dwelt upon here, as the table contains all the information needed for the reader to form his own opinion. It should be stated, however, that the heaviest rabbits of any group were chosen for the bovine cultures. After the inoculation the rabbits were handled very little, in order that the conditions might remain as uniform as possible. For this reason I have no temperature records, but hope to obtain them from a special series of experiments.

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* La tuberculose et son bacille, 377.

The results may be classed as gross or those manifest on ordinary observation and those deducible from a microscopic examination of the diseased tissues and organs. The gross results show a sharp line of demarcation between the bovine and the human cultures. While all the rabbits inoculated with the former succumbed in from 17 to 21 days, of the rabbits inoculated with the latter only one succumbed in 35 days. The others were chloroformed after periods ranging from 1 month and 24 days to 3 months and 18 days. At the time they were killed all but one had increased in weight, some but little, others much.

The rabbits inoculated with bovine bacilli manifested in most cases more or less dyspnœa at the end of 8 or 10 days. The lesions were practically the same in all, with slight minor modifications. The lungs, as the first and chief recipients of the injected bacilli, were in a state of expansion, which in some cases was extreme. All lobes were densely sprinkled over with small grayish tubercles, varying from mere specks to those 1 mm. in diameter. The liver was usually mottled, indicating fatty changes, and in about half the cases it was permeated with barely visible grayish points. The spleen was enlarged to 2 or 3 times its normal size and as a rule permeated with minute tubercles. In the kidneys the visible tubercles were scarce. In 3 cases they were seen in the heart muscle, in several in the follicles composing Peyer's patch at the ileocæcal valve.

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* See Table III, Bovine culture I.

The sputum rabbits, with one exception, behaved quite differently from the bovine cases. A certain number showed signs of illness after 7 or more days. They were quiet, somewhat drowsy and ate little, but manifested no dyspnœa. This condition lasted one or two weeks, after which recovery took place. A certain number did not show this transitory depression. It is probable that with all, during the first month, there was loss of weight, for subsequent weighings showed no gain upon the initial weight. At the time they were chloroformed, they had all more than recovered in weight. The lesions in these rabbits were in many respects quite different from those of the bovine rabbits. The rabbit which received the culture marked Sputum I did not show any recognizable lesions. The difficulty experienced in the attempts to cultivate this bacillus leaves the interpretation of this case somewhat doubtful, although the microscopic preparation from the feeble culture used showed well-stained bacilli. The lesions of the other cases were quite uniform in character.

In all, the lungs contained tuberculous foci sprinkled over the various surfaces from 1 mm. to 1 cm. apart. They did not seem to interfere functionally, for the lungs collapsed normally when the thorax was opened. The tubercles frequently projected somewhat above the surface in the collapsed state of the organ. In the fresh condition the foci were observed as minute dots, barely visible owing to their translucency, as larger foci, 2 mm., in rare cases 3 to 4 mm. in diameter, also translucent excepting the centre, which was occupied by a minute opaque point, and as more diffuse infiltrations of a translucent appearance mainly at the sharp lateral margin of the lobes. These different forms of lesions were usually found associated in the same organ, the largest in the animals kept alive longest. Necrosis was an inconspicuous feature, whereas the translucency was often so perfect as to conceal the small lesions from the eye. When placed in alcohol, these became opaque and visible.

The liver usually contained minute tubercles scattered sparsely over the organ. The spleen, only very slightly larger than normal, was affected to nearly the same degree. The kidneys came next to the lungs in the extent of the lesions. Sprinkled sparingly over the cortex were opaque whitish foci from one to two mm. in diameter. When the organ was incised, these round foci, in many cases, corresponded to radial whitish lines and bands, extending from the periphery towards the pelvis and usually stopping within the medullary portion near its base. More rarely small whitish foci were observed in the walls of the stomach or the large intestine. The lymph nodes were free excepting in one case, in which the node associated with a tuberculous kidney was involved.

In all cases inspection of the affected organs revealed no degenerative changes. They were evidently not disturbed by the focal lesions, an observation strengthened by the increased weight of the animals when sacrificed. The single rabbit which died 35 days after inoculation had received Sputum culture IV. It is worth while to note as a highly probable explanation of this one exception that the rabbit, after inoculation, escaped from the basket in which it was being carried and had to be chased for a long time in the animal room before it could be penned. I at once anticipated some modification of the disease, owing to the different distribution the bacilli would undergo under the influence of muscular exertion. This anticipation was realized. The rabbit after a week became quiet and remained dull and drowsy, without any change in respiration. On the day before death it sat in a crouching position, the hind feet drawn forward under the body, the eyes partly closed. After death its weight was 922 grammes, the initial weight having been 1620 grammes. During the latter weeks a purulent ophthalmia developed in the left eye, and shortly before death there was a slight discharge and beginning opacity of the cornea in the right eye.

In the liver, lungs and kidneys there were minute, translucent tubercles, scarce in the lungs and kidneys, more abundant in the liver. The lesions, found in sections of hardened tissue to be relatively very immature, could not explain the rapid emaciation and death, as they were less extensive than those of the surviving rabbits, unless we assume that the different, more general distribution caused a more rapid breaking up of the bacilli and diffusion of the toxines. Another hypothesis would be the presence of foci in the central nervous system, which was not examined. A second trial upon a rabbit 2 months later, with conditions as nearly the same as possible, proved no exception to the general rule. The rabbit showed but little disturbance, and was chloroformed after 2 months and 9 days. The lesions found were like those induced by the other sputum bacilli.

In order to probe still farther this difference between bovine and human bacilli, the lungs of all but a few rabbits were subjected to a microscopic study. Here the foci may all be considered primary and produced under like conditions. The lesions as defined in fixed and hardened tissue by the microscope in the bovine cases were characterized by rapid necrosis of the tubercle and the presence of very large numbers of tubercle bacilli. This necrosis was evident in the fresh organs from the marked opacity of the comparatively young tubercles. The tubercle itself at the stage in which it came under observation varied slightly from case to case. It consisted of a necrotic centre, absent only in the smallest foci, outside of which a zone of epithelioid cells was distinguishable. This zone was in several cases bounded by a peripheral one of cells having deeply stained nuclei and probably lymphoid. These latter cells were also intermingled with the epithelioid ones. The central necrosis appeared in two forms; it either was characterized by the presence of a very dense, deeply stained mass of nuclear fragments, or else these were present only to a slight degree and the centre had a more rarefied aspect. In the latter case it was evident that the necrotic mass consisted of the bodies of epithelioid cells and the few nuclei present. In the former the source of the great mass of nuclear detritus remains in doubt. These dense nuclear masses were present in abundance in Bov. I and III. In Bov. II and IV they were not quite so conspicuous; in Bov. V least so. It is worthy of note here that Bov. I and III are the two cultures which proved rapidly fatal to cattle inoculated with them, as will be shown farther on.

In the series of rabbits inoculated with human bacilli, the lungs present quite different conditions. The lesions evidently develop very slowly, for in the one rabbit which succumbed after 35 days, the tubercles were very small, translucent, the bulk microscopic in size. With the continued life of the rabbit they grow larger. The microscopic picture differs from that of the bovine series in the rarity of necrosis and in the comparative scarcity of tubercle bacilli. The tubercles consist of aggregations of large epithelioid elements which only in the centre of the larger foci show necrosis. These epithelioid foci may be rarely enclosed by a zone of round cells. Giant cells are present, but as a rule in very small numbers. In the Sputum III rabbit they were exceptionally numerous and very large. The contiguous alveoli may be occluded with patches of epithelioid cells not to be confounded with desquamated epithelium.* Tubercle bacilli are found only in the necrotic centres, either in very small numbers or more abundantly. In some cases they stain feebly. In all cases they are somewhat longer than the bovine bacilli in the same situation.

In addition to the foregoing inoculation tests, made as far as practicable with equal doses, a few others were tried under slightly modified conditions. A large rabbit (No. 20), weighing 2048 grms., received into the abdominal cavity the usual dose, 0.5 cc. of the standard suspension. Death ensued 26 days later. The walls of the abdomen and of the large intestine were densely studded with minute tubercles, the liver permeated with them. The lungs also were densely beset and penetrated with minute translucent tubercles. They did not differ appreciably from the lungs after intravenous inoculation. This mode of inoculation thus proved almost as promptly fatal as the intravenous injection. The same suspension of tubercle bacilli used upon the preceding rabbit was diluted with 9 volumes of bouillon, and 0.5 cc. injected into an ear-vein of a rabbit (No. 27) weighing 1932 grms. The rabbit thus received one-tenth the usual dose. In this animal the symptoms and duration of the disease and the lesions were nearly the same as those of the rabbits inoculated with the horse culture to be described later. The rabbit showed evidences of disease after two weeks, with slowly increasing dyspnœa. On the 34th day it was chloroformed, as it would probably have lived but one or two days longer. The extreme relative virulence of the bovine bacilli is thus put into a clear light.

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* The lesions in rabbits due to sputum bacilli resemble in many respects those produced by Prudden with boiled and washed tubercle bacilli in the same species, if we exclude the slight progressive tendency of the disease due to the living forms. See *N. Y. Med. Journal*, 1891, i, 637.

A similar experiment was made with Sputum culture III. One rabbit received an equivalent of the usual dose, another one-tenth of this on March 1st. Both are still alive (July 20), and the initial weights of 1707 and 1672 grms. are now 1558 and 1986 grms.

It has already been stated that the Nasua culture, presumably derived from sputum, produced neither symptoms nor visible lesions in a rabbit. The swine and the cat culture, however, produced lesions practically identical with those due to the bovine cultures.

The horse culture takes a position midway between bovine and human cultures, so far as the rabbit inoculations show. These, therefore, deserve a more detailed description. The first rabbit, treated like all the rest, remained apparently unaffected for a month, although the weight had fallen slightly from 1505 to 1477 grms. In the second month respiration became somewhat slower and grew gradually more and more labored, so that the animal had to be chloroformed just two months after inoculation. The weight had fallen to 1232 grms. The lesions were macroscopically unlike those in any other rabbit of the series. In the first place, the lungs failed to collapse even slightly. They are densely beset and permeated with tubercles of a grayish, feebly translucent appearance, the majority about 1.5 to 2 mm. in diameter, coalescing into patches 4 and more mm. in diameter. Minute tubercles are also visible among the larger ones. The latter may have a minute, opaque, necrotic centre. The liver is apparently free from tubercles, but the slightly enlarged spleen is beset with sharply outlined, slightly projecting grayish tubercles 1 mm. in diameter. The kidneys are affected in a manner similar to those of the sputum rabbits, but the foci are larger. There are scattering tubercles in the walls of the small and large intestines, but none in the walls of stomach. A second rabbit, inoculated 6 months later, was chloroformed after 40 days on account of severe dyspnœa. The course of the disease and the lesions were like those of the first rabbit. A noteworthy peculiarity of these cases is the presence of tuberculous foci 2 to 4 mm. in diameter in the superficial axillary and popliteal lymph nodes. These were not seen in any of the sputum rabbits, but were produced by retarding the bovine disease in a rabbit. The intermediate position of this horse culture is well illustrated by this experiment referred to above, in which a dilution of bovine tubercle bacilli, equivalent to one-tenth of the usual density employed, produced manifestly the same effect upon rabbits as the horse culture. In other words, the pathogenic power of the bovine culture as measured upon rabbits was 10 times that of the horse bacilli. Further trials will, perhaps, enable us to give a rough arithmetical ratio between the pathogenic power of the bovine and the sputum cultures as affecting adult rabbits. I do not, however, consider the difference between these races to be solely quantitative.

A comparison of the lesions produced by these three races was attempted by a histological examination of the lungs, spleen, liver and kidneys of three rabbits:

No. 16. Wt. 1527–1617: Sput. V: Chloroformed 3 months 7 days after inoculation.
No. 14. Wt. 1540–1232: Horse I: " 2 " 14 " " "
No. 19. Wt. 1443– Bov. V: Died 20 days after inoculation.

I refrain from giving the detailed account of the lesions in each organ and limit myself to the following summary:

Sputum V. Homogeneous epithelioid cell foci of small size, with very little central necrosis and very few tubercle bacilli. In the lungs and kidneys these foci have lymphoid cell mantles, in the liver well-defined connective tissue capsules.

Horse I. Large foci in lungs, where they are so numerous as to coalesce; some large foci in kidneys; smaller ones in the spleen. In the liver the foci are represented simply by one or several large giant cells, situated within the lobules. Foci large in subcutaneous lymph nodes and Peyer's patch. Necrosis of the foci quite extensive in all the organs excepting the lungs, where it is slight. Tubercle bacilli fairly abundant in all foci.

Bovine V. Large numbers of epithelioid cell foci in lungs, liver and spleen. In the latter epithelioid cells are also abundant throughout the pulp. Foci small in kidneys. Necrosis under way in lung foci. Tubercle bacilli very abundant in all foci.

Taking into consideration the time during which these lesions developed, we may epitomize the observed differences as follows:

In the first rabbit, very gradual development of the tubercles, with suppression of the bacilli and reparative changes leading to a stationary condition of the disease. In the second rabbit, continuous but not very rapid development of tubercles in the various organs. In the kidney and spleen evidences of limitation of the foci. No evidence that bacilli are repressed. In the third rabbit very rapid appearance of the foci, with unchecked multiplication of the bacilli and rapid onset of necrotic changes.

As a matter of some interest there may be briefly mentioned certain lesions of the eye in three of the sputum rabbits. In rabbit No. 7, inoculated with Sputum culture IV, which animal was the only one of all the sputum rabbits to succumb (in 35 days), a purulent ophthalmia appeared in the left eye some time before death. Soon after a slight cloudiness of the cornea of the other eye was noticed. A discharge had started in this shortly before death. I did not associate these lesions directly with tuberculosis, although later cases make it probable that the purulent discharge was secondary to tuberculosis of other parts of the eye. At the autopsy I examined the discharge for tubercle bacilli, but found no acid-resisting bacilli.

The second rabbit (No. 12) inoculated with a culture from the same source first called my attention to this localization of the tubercle bacilli. In this animal there was noticed some weeks after the inoculation, a slight nodular thickening of both lids of the right eye, which slowly grew more conspicuous. Vision became impaired by the enlargement and gumming together of the lids, so that the rabbit used the other eye only. When this animal was chloroformed, 3 flattish, disk-like, firm excrescences were found on the sclera at the margin of the cornea, about 90° apart. Of these, as well as of the nodulated lower eyelid, sections were made, and both lesions proved to be tuberculous. In both the tubercle bacilli were sparingly present. The neoplasm in the eye-ball had involved nearly the whole thickness of the sclera. In the eyelid the multiple foci were external to the lobules of the Meibomian glands. In still another animal eye lesions were found. About 2½ months after rabbit No. 16 had been inoculated with Sputum culture V, the lids of the left eye were observed to be gummed together. A pasty, yellowish exudate was found under the lids and the cornea was slightly opaque. About 3 weeks later the animal was chloroformed and faint whitish spots were seen in both eyes, presumably on the iris. Sections of the eye hardened unopened in Zenker's fluid confirmed this supposition. Small epithelioid tubercles were found in the vascular stroma of the iris as well as in one ciliary body. Linear infiltration of the cornea was observed, starting from the most affected side and extending to the middle of the cornea. In its course several sections of vessels were found. A similar infiltration had begun from the opposite side. In the foci tubercle bacilli were very scarce. The infection probably came through the blood, as infection from the point of injection on the ear is hardly supposable, since the lesions here are of the slightest character. The animals had been kept separated, and could not have acquired the infection from the exterior. The eye of the rabbit thus seems to be a very vulnerable organ, and earlier experimenters have, in a way, chosen by accident that organ in this species most likely to give positive results.*

The divergences noticed when rabbits weighing between 1300 and 2200 grammes are inoculated into an ear-vein with 0.5 cc. of a well-clouded suspension of tubercle bacilli in bouillon are as follows:

1. Death of the bovine cases in 17 to 21 days.
2. Rapid evolution and necrosis of the pulmonary tubercles, with very great increase of the tubercle bacilli in them.
3. Death of the sputum rabbits (one explainable exception) did not ensue, but after 1½ to 3½ months the original weight had been more or less exceeded.
4. The pulmonary tubercles in the sputum rabbit developed very slowly, with very little tendency towards necrosis. The bacilli were present in very small numbers only.
5. The swine and cat cultures are to be classed with the bovine, the horse culture stands intermediate.

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* The eyes of Sputum rabbit VII were affected in the same manner.

TABLE III.—RABBITS.

Culture.	Total age of artificial culture.	Number of transfer.	Date of inoculation.	Age of culture used, in days.	Dose in cc.	Mode of inoculation.	Designation of rabbit.	Initial weight in grammes.	Final weight.	Chloroformed after	Died in
Sputum I	10 mos. 27 days.	2nd	June 16, 97.	61	0.75	ear-vein.	17	1470	1681	2 mos. 3 days.	
" II	3 " 3 "	5th	Feb. 24, 97.	24	0.5	" "	6	1380	1645	1 " 26 "	
" III	1 " 19 "	2nd	April 3, 97.	18	0.5	" "	8	1790	1830	2 " 8 "	
	13 " 17 "		Mch. 1, 98.	16	1.0‡	" "	28	1707		Alive July 20; weighs 1558.	
	13 " 17 "		" " "	16	1.0*	" "	26	1672		Alive July 20; weighs 1986.	
" IV	1 " 18 "	2nd	April 3, 97.	18	0.5	" "	7	1620	922		1 mo. 5 days.
	4 " ——	5th	June 16, 97.	12	0.5	" "	12	1620	1722	2 mos. 9 days.	
" V	1 " 19 "	3rd	Aug. 26, 97.	10	0.5	" "	16	1517	1617	3 " 7 "	
" VI	1 " 12 "	3rd	Aug. 26, 97.	10	0.5	" "	18	1305	2061	3 " 18 "	
" VII	1 " 10 "	2nd	Mch. 1, 98.	16	1.0†	" "	29	1882	1788	3 " 8 "	
Bovine I	15 " 19 "	14th	Mch. 20, 96.	16	0.5	" "	3	2279	——		17 days.
	37 " 24 "	38th	Jan. 25, 98.	12	0.5	" "	24	1795	——		22 "
" II	2 " 27 "	3rd	Feb. 24, 97.	24	0.5	" "	5	1360	——		17 "
" III	4 " 7 "	5th	Aug. 26, 97.	10	0.5	" "	15	1775	——		21 "
" IV	4 " 3 "	5th	Aug. 26, 97.	10	0.5	" "	17	1607	——		15 "
	9 " 23 "	9th	Feb. 15, 97.	17	0.5	abdomen.	20	2048	——		26 "
	9 " 23 "	"	" " "	17	0.5§	ear-vein.	27	1932	1416		Killed on 34th day, very severe dyspnœa, probably within 24 hours of time of death.
" V	4 " 3 "	5th	Aug. 26, 97.	10	0.5	" "	19	1443	——		20 days.
" VI	2 " 18 "	3rd	Mch. 15, 98.	13	1.0†	" "	21	2353	1877		Chloroformed on 22d day; dying.
Nasua	19 " 25 "	18th	Mch. 20, 96.	16	0.75	" "	2	1800	fat condition wt. ?	1 mos. 24 days.	
Swine I	2 " 6 "	4th	July 14, 96.	12	1.	" "	1	2000 (?)	——		17 days.
Horse I	5 " 4 "	6th	July 24, 97.	18	0.5	" "	14	1540	1232	2 " 4 "	
	11 " 5 "	11th	Jan. 25, 98.	12	0.5	" "	25	1653	——	1 " 9 "	
Cat I	10 " 17 "	5th	Jan. 25, 98.	18	0.75	" "	13	1320	——		20 "

* Dilution about one-twentieth of the standard. † Dilution about one-fourth of the standard. ‡ Dilution about one-half of the standard. § Dilution about one-tenth of the standard.

Gray Mice. White mice have been pronounced quite insusceptible by Koch. Of 5 inoculated subcutaneously with a culture from the monkey, 12 with a culture from miliary tuberculosis, only one of the first set showed a few grayish nodules in the lungs. On the other hand, field-mice were found quite susceptible.

Two parallel tests were made upon gray house-mice with cultures of Bovine V and Sputum VI. At the time control inoculations of the suspensions used were not made upon guinea-pigs, and subsequent developments made it doubtful whether Bovine V was alive. This part of the experiment is therefore omitted from the table given below and a later experiment with Bovine VI substituted. The suspension used in this latter test was employed simultaneously upon 2 guinea-pigs and a rabbit with the usual success.

In the first bovine test the 4 mice inoculated, 2 subcutaneously and 2 into the abdomen, all died, the first on the 8th and the last on the 37th day, without any lesions recognizable with the naked eye. In the cases of the 4 mice inoculated with the sputum culture, death ensued between the 8th and the 40th day. In one of these the lungs contained a number of whitish, rather firm, nodules, 1 to 2 mm. in diameter. In sections these were found to consist of completely necrotic foci, enveloped by a dense, broad zone of pneumonic infiltration, within which all alveoli were filled with nuclear debris. A striking feature were the immense numbers of rather slender, mainly beaded tubercle bacilli, which occupied not only the necrotic centre, but in equal abundance the pneumonic periphery. This case proved the vitality of the sputum culture, and the test is therefore inserted in the table below.

TABLE IV.—GRAY MICE.

Designation of culture.	Total age of culture.	Number of transfer.	Age of culture used.	Dose and mode of injection.	Results.
Sputum VI	5 mos. 27 days.	6th	24 days	Mouse 1, 0.5 cc. subcut...	Dies in 34 days, no tuberculosis.
				" 2, 0.5 cc. " ...	Dies in 40 days, no tuberculosis.
				" 3, 0.25 cc. abdomen.	Dies in 8 days, no tuberculosis.
				" 4, 0.25 cc. "	Dies in 34 days, tubercles in lungs.
Bovine VI	2 mos. 18 days.	3rd	13 days	" 5, 0.25 cc. subcut..	Chloroformed in 37 days
				" 6, 0.25 cc. " ..	" " "
				" 7, 0.25 cc. abdomen	Dies in 5 days.
				" 8, 0.25 cc. "	" " 5 "

The second bovine test resulted in the death of the two mice receiving the dose into the abdomen, in 5 days. There were no lesions recognizable. The two mice inoculated subcutaneously were active up to the 37th day, when they were chloroformed. Lesions were not found.

Pigeons. Fowls and pigeons have been used with success in distinguishing avian from mammalian tubercle bacilli. Hence it was thought best to test human and bovine bacilli on pigeons. The cultures used are given below in the table. The bacilli, in the form of bouillon suspensions, were injected into the pleuro-peritoneal cavity, the needle being introduced caudad of the sternum. In one case the injection was made into a wing-vein. The inoculations were in general negative, with the possible exception of that of the swine culture. In this bird there was found hyperæmia of the serosa of the intestines, with an eruption of scattering, minute grayish dots. There were similar opaque points on the air-sac membranes. On the duodenal loop were found some particles of viscid exudate composed exclusively of coarsely granular cells. In this exudate some tubercle bacilli were still recognized by the specific stain. In some of the other pigeons there were a few opacities of the mesenteries and air-sac membranes, with perhaps one or more conspicuous Peyer's patches. Even in refractory animals, lesions such as these might be anticipated where the tubercle bacilli are deposited.

TABLE V.—PIGEONS.

Designation of culture.	Total age of culture.	Number of transfer.	Age of culture used.	Designation of pigeon.	Dose and mode of injection.	Remarks.
Sput. II	6 mos. 14 d.	9th	11 days	No. 2	1 cc. abdomen...	Chloroformed in 45 days: negative.
" III	3 " 19 "	5th	11 "	" 4	1 cc. " ..	Chloroformed in 57 days: negative.
Bov. II	5 " 26 "	9th	11 "	" 1	1 cc. " ..	Chloroformed in 49 days: negative.
Swine I	1 yr. 19 "	17th	11 "	" 3	1 cc. " ..	Chloroformed in 57 days: minute excrescences on portions of intestine and membranes of air-sacs.
Sput. V.	5 mos.	5th	31 "	" 5	¾ cc. wing-vein..	Chloroformed in 47 days: negative.
Bov. V	8 mos. 14 days	8th	24 "	" 8	1.5 cc. abdomen..	Chloroformed in 49 days: negative.
Horse I	11 mos. 5 d.	11th	12 "	" 7	0.5 cc. "	Chloroformed in 50 days: negative.

Cattle. It is a noteworthy fact that, in spite of the great economic, agricultural and hygienic interests involved in the diffusion of bovine tuberculosis throughout the world, no experiments upon cattle with cultures of the bovine bacillus had been made up to the time of my first experiments, published in the *Transactions of the Association of American Physicians* for 1896. Experiments upon cattle with the human bacillus had been made by several observers. A discussion of these tests I shall defer to the end of this article, because of certain secondary questions, which will be clearer when my tests have been described.

The results of the inoculation of two heifers into the lungs with Bovine culture I and *Nasua* culture have already been published.* It may be stated briefly that the bovine culture produced general tuberculosis, ending fatally in 35 days, while the Nasua (human) culture failed to produce any recognizable lesions.

During the warm season of 1897 inoculations were made with 5 sputum, 1 swine and 4 bovine cultures upon as many cattle. For these 10 animals I am indebted to the Massachusetts Board of Cattle Commissioners, who have shown much interest in the progress of these investigations, because of the need felt by them of more definite information upon the relation of bovine tuberculosis to the human disease. The test was carried out in two series, the first including 4, the second 6 animals. The culture material used was suspended in bouillon, as described, the density of the suspension being about the same as that of a typhoid culture in bouillon 24 hours old. In both series the dose was 2 cc. of such a suspension.

Series I. The 4 animals of this series were inoculated with the following cultures:

Sputum II,	total age	5	months	9	days ;	8th	transfer.
Sputum III	"	2	"	15	"	4th	"
Bovine II,	"	5	"	2	"	5th	"
Swine I,	"	11	"	23	"	16th	"

The swine culture is probably bovine in origin, and it was used on this supposition. Assuming that tubercle bacilli become slowly weakened in virulence by artificial cultivation, we should endeavor to use cultures as fresh and of as nearly the same age as possible. This theoretical demand cannot be successfully met, because of the many difficulties surrounding such work. Of the 4 cultures used, Sputum II and Bovine II are of nearly the same age, while Sputum III is but half as old as they, and Swine I more than twice as old.

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* Loc. cit.

In making the injection, the space between the 6th and the 7th rib was chosen. The needle was inserted about 3 inches above the level of the elbow (olecranon process). It was found subsequently, at the autopsy, that the point chosen was too low, and that in all of the animals the needle, leaving the lungs intact, pierced the diaphragm. Some of the bacilli were discharged into the muscles of the thoracic wall, some into the abdominal cavity and some into the pleural cavity. Bearing this accident in mind, we may now go to a description of the further history of the inoculated cattle. They were all housed in a spacious, well-ventilated barn in large commodious horse stalls. A piece of ground adjoining the barn was enclosed, and in this the animals spent 6 to 7 hours a day for about 4 weeks. Thereafter the animals were separated into two lots, one lot being out several hours in the morning, the other several hours in the afternoon. The two which received the bovine and the swine culture were allowed to run together, similarly the two which received the sputum cultures. It might be claimed that there was in this arrangement a possible danger of transmitting the bacilli from one animal to another and of infecting the ground. There was no evidence of this at the post-mortem examination, and the arrangement was considered safe at the start because it takes some time for the tuberculous tissue to become disintegrated. It was, however, deemed prudent not to keep the animals longer than 2 months, on account of the imperfect isolation.

In apportioning the cultures to the animals, the sputum cultures were injected into the youngest animals, in order that these cultures might have any advantage likely to accrue from difference in age. Two yearlings (without any permanent incisor teeth) received the two sputum cultures, the bovine culture was injected into a heifer about 2½ years old, the swine culture into a heifer about 2 years old.

They were killed and examined at the Brighton (Boston) abattoirs, with the co-operation of the Board of Cattle Commissioners, just two months after the day of the injection. Before injection, these animals had been tested with tuberculin by the Board and found free from tuberculosis. Still, since this agent occasionally allows an animal to escape which contains foci of the disease, attention was directed to this point at the autopsies. No lesion, however, was found which from its situation and appearance could be referred to any former spontaneous infection.

Let us examine first the effect of Sputum culture II and Bovine culture II, which were of nearly the same age when injected.

The weight of the yearling which had received the sputum culture had risen in the two months from 520 to 580 lbs., that of the heifer from 650 to 710 lbs. There was no continuous fever recognized in either animal, though the temperature was taken twice a day, morning and afternoon. The fluctuations noticed were evidently due to the effect of the sun while the animals were in the enclosure.

The lesions in the yearling were very slight. At the seat of inoculation between the 6th and 7th ribs a mass of tubercles attached to pleura about 1 x ½ inch in dimension, the tubercles composing it partly cheesy, partly firm. Near the cephalic border of ventral lobe of right lung, a subpleural nodule, not yet necrotic, about ⅛ inch in diameter. On abdominal aspect of diaphragm, right side, about 24 isolated tubercles 1/12-⅛ inch in diameter. A few similar tubercles on the omentum, which is slightly adherent to the large intestine. When adhesion was removed, about 6 or 7 nodules found on caecum 1/12 inch in diameter. Evidently the injection needle had passed through the diaphragm into abdomen, depositing some fluid there and some in thorax when partly withdrawn.

The lesions in the heifer which had received the bovine culture were quite extensive and were diffused through thorax and abdomen, owing to the penetration of the diaphragm by the needle of the injection syringe:

Thorax. The right pleural cavity shows an abundant eruption of tubercles along the borders of the ribs. Some of the masses formed are characteristically flattish, grape-like and in bulk quite large. One mass measured 8½ x 3 x 1½ inches. Others of similar dimensions were present. On the lateral margin of the right lung a series of loosely attached, flattish neoplasms up to 2 inches in diameter, besides hyperæmic fringes of loose connective tissue. On the convex surface of this lung only a few tubercles. Large patches of tubercles on pericardium and diaphragm.

In the muscular portion of diaphragm, right side, a mass of tuberculous tissue (probably place where needle penetrated) 1½ x 1½ x ½ inch in dimension. The caudal of the series of dorsal mediastinal glands about twice normal size on account of the presence of many small foci, showing in some cases an opaque, yellowish centre.

Abdomen. The omentum densely studded with agglomerations of tubercles covering the greater part of its surface. These masses vary up to ½ inch in thickness. Similar patches on abdominal aspect of diaphragm and on spleen. Fewer patches on gall-bladder and on liver. In one of the portal glands a ¼-inch focus, pale, grayish, permeated with small calcareous spicules.

The examination of sections of the pleural eruptions and of one of the dorsal mediastinal glands showed the typical lesion of bovine tuberculosis, with its foci of epithelioid cells and its numerous giant cells containing a few tubercle bacilli. Any detailed description of these lesions would be simply a repetition of familiar facts.

We have in these cases a wide divergence in the result of inoculation. The human bacillus produced a slight eruption of small, tubercle-like bodies, which did not present even microscopically the characters of true tubercles, while the bovine bacillus produced an exquisite case of pearly disease both in thorax and abdomen, with the formation of large, grape-like masses in the chest.

The youngest sputum culture (III) was injected into a yearling weighing 410 lbs. At the end of two months the weight had risen to 480 lbs. The lesions found are slight:

On the abdominal aspect of diaphragm, right side, a patch of isolated tubercles about 2 inches in diameter, the tubercles themselves about ⅛ inch in diameter and about ½ inch apart. They are grayish, opaque. At place of inoculation, on serous aspect of ribs, a flattish neoplasm 1 inch in diameter and about ⅛ inch thick. Other lesions not detected. In this case also the needle evidently entered abdomen through diaphragm. Sections made from the small isolated tubercles on the abdominal surface of the diaphragm show them to be masses of newly forming tissue. They consist in the main of spindle-shaped connective-tissue cells running in parallel and interlacing groups and richly supplied with vessels. No trace of the characteristic tubercle formation or of bacilli, although these bodies are evidently due to the bacilli injected.

The swine culture was injected into a somewhat older animal weighing 620 lbs. After two months the weight was 660 lbs. The autopsy showed the following conditions:

At place of inoculation (right chest wall) a subcutaneous tumor about 2 inches in diameter, made up of a very dense connective-tissue sac, ¼ inch thick, which encloses a pale yellowish semifluid mass (caseous). There are besides this focus, in the same situation, 3 smaller nodules from ½ to ¾ inch in diameter, the largest with caseous centre. On the pleural aspect a similar ½-inch centrally softened focus.

In nearly the centre of the right half of diaphragm and projecting into abdomen for ½ inch is a tumor representing portion of a larger focus in the muscular portion of the diaphragm about 1 inch in diameter. This focus is likewise caseous, diffluent centrally. On all the ribs of right pleural cavity are eruptions of small tubercles, reaching in some instances a diameter of ⅛ inch. Besides the palpable tubercles, there is along one border of each rib a line of vascular fringes of connective tissue. In many of these fringes tubercles are not noticed, although the fringes themselves are evidently a result of the injection.

Along the lateral border of the right lung similar vascular fringes of tissue containing tubercles. These fringes extend dorsad for about 2 inches on ventral and cephalic lobes.

Diffuse eruption of minute tubercles on pleural aspect of diaphragm, right half.

Large caudal mediastinal gland contains large numbers of tubercles, varying in size from mere points to those ⅛ inch in diameter and showing beginning necrosis. Left bronchial gland several times normal size. Ventral mediastinal gland several times normal size and containing several large, centrally caseous foci. One mesenteric gland contains several small necrotic tubercles.

In this case many of the bacilli had been deposited in the fleshy portion of the diaphragm and some under the skin, and had thus been prevented from exerting their greatest power. Sections were made of the subcutaneous foci, the anterior mediastinal and bronchial lymph-glands and of the vascular fringes on the pleural aspect of the diaphragm. The glandular and subcutaneous lesions are characteristically tuberculous, with a greater tendency towards necrosis and disintegration of the tubercles than in the animal inoculated with Bovine culture II. The lesions of the serous membranes are more vascular and show greater tendency toward the formation of connective tissue around the minute tubercles. In general this culture must be considered less potent than the bovine culture. Whether this is due to the more prolonged artificial cultivation or to the species through which the bacillus had passed remains unsettled.

Series II. In this experiment three sputum and three bovine cultures, recently isolated, were used. The bovine cultures were of nearly the same total age. The sputum cultures differed somewhat from them and from one another in this respect.

Sputum IV,	total age	6 months	10 days,	7th transfer.
" V,	"	1 month	19 "	3rd "
" VI,	"	1 "	12 "	3rd "
Bovine III,	"	4 months	7 "	5th "
" IV,	"	4 "	3 "	5th "
" V,	"	4 "	3 "	5th "

The cultures used for the injection were all 9 days old, grown on the same lot of dog's serum. Control inoculations of guinea-pigs and rabbits will be found on tables II and III. The animals at my disposal, unfortunately, varied considerably in age, and in assigning the cultures the advantage was given to the human cultures:

Sputum IV, yearling, 525 lbs., no permanent incisor teeth.
Bovine III, " bull, 645 lbs., no permanent incisor teeth.
Sputum V, cow, 675 lbs., about 4 years old.
Bovine IV, " 850 " " 12 ".
Sputum VI, cow, 865 lbs., about 3½ years old.
Bovine V, " 875 " " 6 "

The injection of the cultures was carried out as in Series I, excepting that the point of insertion of the needle was chosen higher up, about 11 inches above the elbow of the animal when in the standing position, between the 7th and 8th ribs. The length of the needles used was about 2 inches. The care of the animals was the same as that bestowed on the preceding lot, excepting that the bovine and the sputum animals were kept separate in the out-door enclosure from the start, the one lot being out in the morning, the other in the afternoon. They were kept two months, with the exception of the young bull, which died 17 days after the inoculation. The four cows of this lot gave at the start altogether about 8 quarts of milk. The secretion was slowly dried up, so that in the sixth week a very little, amounting perhaps to one quart in two days, was removed. The milking was continued chiefly to prevent any udder troubles during the experiment and to maintain normal conditions. The temperature was taken but once a day, at noon.

In comparing the temperature records of these 6 animals it was noticed that the three animals which received the bovine cultures had a high temperature immediately after the inoculation, which lasted until the death of the bull and about three weeks for the remaining two animals. At the same time no such elevation of temperature was recorded for the animals receiving the three sputum cultures. There was but one well-defined rise of temperature in the case of the yearling, from the 13th to the 16th day after inoculation. The other irregularities are probably due to the fact that the temperature was usually taken after these animals had been in the enclosure in the sunshine for several hours. Those with the high temperature were kept much of the time in the cool barn in the morning, which probably depressed the fever curve somewhat. After the period of fever, no other elevations were noted up to the close of the experiment.

The young bull, inoculated with Bovine culture III, showed besides the prompt onset of a high temperature, general and local disturbances about a week after the inoculation. The breathing became rapid, the appetite had partly gone. Emaciation and weakness supervened. He was unable to get up September 11, and died the following night.

The autopsy revealed a severe miliary tuberculosis of both lungs with marked congestion and œdema of the organ. Normal collapse no longer possible. The associated lymph-glands were much enlarged and infiltrated with minute tubercles. Patches of minute tubercles were found on the pleural covering of ribs and on the omentum. Sections from the lungs, dorsal mediastinal glands, pleural neoplasms, liver, spleen and kidneys were examined. In the lungs, mediastinal glands and pleural neoplasms, tubercles were very numerous, in the liver less abundant. In the kidney sections none were seen. In these different situations the tubercles presented nearly the same peculiar features, which distinguished them readily from the tubercles of the spontaneous disease in cattle. To make the differences clearer, a brief description of the lesions in a spontaneously diseased lymph-gland may not be out of place.

A rarefied area within the follicle containing a few nuclei having the characters of endothelioid nuclei and belonging to what are generally known as epithelioid cells, which are imbedded in or whose outlines are fused with a matrix appearing either homogeneous or else very delicately reticulated. In larger foci this rarefied area contains also one or more giant cells whose body is frequently seen merging by prolongations into the reticulated matrix of the tubercle. Among the epithelioid nuclei are isolated nuclei closely resembling those of lymphoid cells. Tubercle bacilli are, as a rule, scarce and situated usually within giant cells. The largest foci are centrally necrotic, that is, they contain a coarse trabecular matrix staining with eosin and picric acid and containing scarcely any nuclei and very little, if any, fragments of nuclear origin. In this mass tubercle bacilli are rarely found. In the lesions of the bull the tubercles have in the first place no giant cells. The new tissue appears to be made up of cells resembling the usual epithelioid cells of tubercle, but much more crowded together and with less definition of the cell outlines. The nuclei are mostly distorted, drawn out, band-like or horse-shoe-like, and twisted into various bizarre forms. Condensation and fragmentation of the nucleus are also seen. In the tubercles in all situations much fibrin is demonstrated by Weigert's stain. This is especially distinct in the liver tubercles. Here the intercellular substance from material hardened in alcohol stains deeply in eosin. In sections subjected to Weigert's fibrin stain, this substance is blue, appearing throughout the tubercle both as a delicate mesh and as very coarse trabeculæ. In material hardened in Zenker's fluid the fibrin stain is less pronounced. In all the tubercles, large numbers of tubercle bacilli are present. These differences are probably due to the rapidity of the process.

On October 27, the remaining five animals were killed at the Brighton abattoirs, with the co-operation and assistance of the Board. The three sputum animals had all gained in weight:

Yearling (Sputum IV) from 525 to 610 lbs.
Cow (Sputum V) from 675 to 750 lbs.
Cow (Sputum VI) from 865 to 960 lbs.

Sputum culture IV. One permanent incisor on the right has appeared since date of inoculation. In utero a fœtus about three months old. At point of inoculation in the subcutaneous tissue a small nodule about ¼ inch in diameter with contents soft, cheesy. Attached to this is another, smaller nodule about ⅛ inch in diameter.

On the right side of chest wall, pleural aspect, there are attached along the six caudal ribs, soft dark red, pendulous masses of newly-formed, highly vascular connective tissue. At point of inoculation, between 7th and 8th ribs, a flattish pediculated mass of tissue of brownish red color. On the 10th rib another mass about ⅜ inch in diameter. The left side of thorax is normal.

Right lung. On the smaller (cephalic and ventral) lobes, newly formed, delicate fringes of hyperæmic connective tissue, which appears also along the free lateral margin as a delicate band about ½ inch broad, as well as on a portion of the convex surface of the lung, occupying exclusively the lines representing the boundaries of the lobules.

In the large caudal lobe, which is similarly beset with the vascular fringes, a tumor, representing the place where needle penetrated the lung tissue, projecting slightly above the convex surface, is found two inches from the caudal tip. This tumor, about ¾ inch in diameter, contained a completely disintegrated mass and about a dozen surrounding foci, 1/16 to ⅛ inch in diameter, with yellow softened centres. On the margin of this same lobe, in addition to the vascular fringes, are four firm masses of grayish tissue, smooth, flattish, attached by pedicles to the margin of the lobe. The diameters of the largest are ¼ and ½ inch.

On large (caudal) lobe of left lung there is only a very little development of vascular fringes. Imbedded in the same lobe near lateral margin is a uniformly grayish, slightly translucent mass, sharply defined from the enveloping normal lung tissue. Sections of this focus showed a peculiar lymphoid structure of the tumor, in the centre of which a parasite was lodged. Not

having any relation to the inoculation disease, it need not be further discussed.

Attached to the cephalic lobe of the left lung by a pedicle is a small, flattish, smooth mass of new tissue.

The pleural aspect of diaphragm and portions of the pericardium are covered with areas of the highly vascular neoplastic tissue. In some, small nodules can be felt at the free extremity of this tissue.

On the right ventricular surface of the heart four flattish, pediculated masses about as large as split peas are attached.

The various lymph-gland systems, ventral and dorsal mediastinal and bronchial glands, do not show the presence of tubercles or any augmentation in size.

Of this case, sections of the primary lung focus, of two of the pediculated bodies (one from the lung, one from the rib) and of the vascular fringes from the lateral border of the lungs were examined.

The small foci immediately bordering the disintegrated primary focus vary from 0.2 to 2 mm. in diameter. The smallest do not differ essentially from ordinary tubercles, excepting in the scarcity of giant cells. The larger ones are centrally necrotic, with a marked tendency towards encapsulation on the periphery by the condensation of circularly disposed, fusiform, connective-tissue cells. In the smallest tubercles, bacilli are scarce, in the largest they are numerous within the necrotic centre, where they appear as slender, often bent, sometimes fragmented and rather feebly stained rods. In the surrounding epithelioid zone a few well-stained bacilli are present. Passing from the primary place of deposit, the only other lesions are those of the pleura. These occur in two forms, as vascular fringes and as firm, smooth, pediculated bodies, some flattish, like pumpkin seeds in size and outline.

The vascular fringes occur: 1. As mere endothelial tubes; 2. As such enveloped by a layer of wavy fibrillar tissue and covered with a layer of endothelial cells; and 3. As vessels sheathed with a zone of round cells, proliferating endothelial and connective-tissue cells in addition to the fibrillar tissue. Acidophile, polymorphous leucocytes are present here and there throughout the granulation tissue. Mitoses are occasionally seen. After a prolonged search through 7 sections, one minute rarefied area is detected. In it evidently a tubercle bacillus with long axis nearly at right angles to the section.

The vascular fringes from the thoracic aspect of the diaphragm are like those from the lungs. In the former no signs of tubercle formation.

Sections from one of the neoplasms attached to the ribs show two fairly distinct regions, an outer of dense connective tissue and an inner made up of foci of lymphoid elements and a central necrotic core. This latter body was evidently a vascular offshoot of the pleura, which, before necrosis, had attached to or imbedded in it many lymphoid cells, for the necrotic core contains much nuclear matter traceable to such cells. This core is partly enveloped by a ring of epitheliod cells with nucleus at the outer free end, like those around a foreign body. Some are multinuclear and resemble giant cells. In the zone immediately outside of this cellular ring are several characteristic giant cells. There are no tubercle bacilli, nor is there any tissue recognizable as epithelioid and identifiable as tubercle.

Sputum culture V. Cow about 4 years old. In utero a fœtus 3 to 4 months old.

Development of vascular fringes along one border of ribs of the right side of thorax as in preceding case, but amount relatively slight. In the intercostal muscles at the point of inoculation a mass of perhaps a dozen small grayish tubercles.

In the large caudal lobe of the right lung, in the same situation as in preceding case, a projecting tumor about 1 inch in diameter. When incised it is found composed of two ½-inch foci of disintegrated, cheesy-viscid matter enclosed in thin, smooth capsules. No surrounding infiltration. Along the margin and on the caudal surface of this lobe, slight development of pendulous vascular tissue and a sessile tubercle about 4 mm. in diameter. On the left caudal lobe only very slight production of vascular tissue.

On the surface of one of the middle dorsal mediastinal glands an aggregation of minute tubercles, the whole about ½ inch in diameter. Imbedded in the cortex of the same gland two minute tubercles.

In this animal the disease of the middle mediastinal gland, although very slight, is shown in sections to be tuberculosis. The lesions are not to be distinguished from those of the spontaneous disease in cattle, excepting in the presence of considerable numbers of tubercle bacilli. The tubercles themselves contain the usual number of giant cells, and the largest ones are centrally necrotic, the necrosed mass being indistinctly fibrillar in character, staining deeply with eosin and devoid of nuclear debris. The bacilli in this mass stain rather feebly when compared with the deep stain of those in the giant cells on the periphery of the tubercle. This lesion will be again referred to in summing up the experiments.

Cow (Sputum culture VI). In utero a fœtus about four months old.

Within the thorax on the right side, between the 7th and 8th ribs, is a small, pediculated, blackish (hæmorrhagic) mass of firm tissue about as large as a pumpkin seed, another on the 10th rib. On most of the ribs behind the 7th are gelatinous-looking pendulous vascular fringes of neoplastic tissue. On the pleural surface of the diaphragm a similar development of vascular tissue and several firm, pediculated masses like those on ribs.

Of the right lung the cephalic lobe shows very slight formation of marginal fringes. In the ventral lobe whose tip is adherent to the pericardial fat, a uniformly grayish, sharply defined focus ¼ inch in diameter.

In the same situation as in the preceding cases the caudal lobe shows a slightly projecting tumor about 1¾ inches in diameter externally. When incised it is found to consist of a smooth-walled sac 1¼ inches in diameter, containing a yellowish curdy mass, together with a little turbid fluid. No surrounding infiltration. On the convex surface of this lobe there is a slight growth of vascular tissue. Near the caudal tip a flattish mass, partly yellowish, partly blackish, attached by loose tissue to the margin of the lobe. It is about 1 inch long and ½ inch thick.

In the abdomen, a flattish, sessile mass of pinkish-gray tissue about ¾ inch in its longest diameter, attached to omentum.

In this animal, sections of the grayish focus in the ventral lobe of the right lung and of the suspicious sessile mass on the omentum demonstrate the fact that both lesions are parasitic in origin and due to the same cause as the lung focus in cow inoculated with Sputum IV. Both foci are made up of spherical agglomerations of lymphoïd cells, in the centre of which the parasite is situated.

The largest of the pendulous masses from the lungs was examined microscopically and found to be similar in structure to those already described. It consists chiefly of a connective-tissue stroma of interlacing fibres in which are imbedded many capillaries. Scattered through the inner half of the mass are quite small foci of epithelioid and lymphoid cells, but giant cells are absent. In these foci, a few tubercle bacilli are found. The periphery of this pendulous mass consists of circularly condensed connective tissue, beneath which is a homogeneous, somewhat reticulated substance, staining well with eosin and enclosing a few tubercle bacilli. It is probably a layer of fibrin undergoing hyaline degeneration. Small areas of it are still stainable with Weigert's fibrin stain.

Of the three animals receiving bovine tubercle cultures, the fate of one (young bull No. 71) has already been given. The two other cases remained stationary in weight:

No. 88—original weight 850 lbs.; weight at end of experiment 850 lbs.

No. 63—original weight 875 lbs.; weight at end of experiment 870 lbs.

The autopsy notes are in brief as follows:

No. 63 (Bovine culture V). White cow, spotted with red. Horns sawed off. Probably six years old. Fœtus in utero about two months old.

Thorax. Right lung adherent to chest wall in several places. At point of inoculation, between 7th and 8th ribs, an excrescence of the costal pleura about ¾ inch in diameter, of dense, pearly-looking connective tissue enclosing a disintegrated mass. Numerous masses and aggregations of small tubercles on all ribs. These masses in some cases are several inches in length. The left side of thoracic wall, below the level of the point of inoculation on the opposite side, is covered with a uniform, pinkish-gray deposit of very minute tubercles. Eruptions of tubercles on pleural surface of diaphragm and on pleural covering of dorsal mediastinal space.

On caudal lobe of right lung a considerable number of tubercular masses, flattish, sessile, from $\frac{1}{8}$ to $\frac{3}{4}$ inch in diameter. Between the cephalo-lateral border of this lobe and the pericardium is a mass of newly formed tissue, dense, in which are imbedded many minute yellow tubercles and masses of pericardial fat. The whole is about as large as a fist. It binds the lung tissue, pericardium and diaphragm together. Many tubercles on the caudal surface of this same lobe. There is no distinct focus in this lobe as a result of the injection, and it is probable that much of the fluid was deposited in the pleural cavity. But palpation reveals throughout both lungs small shot-like bodies in close proximity. On section numerous yellow tubercles from $\frac{1}{32}$ to $\frac{1}{16}$ inch in diameter are found imbedded in the lung tissue of all lobes.

The dorsal mediastinal lymph-glands are all several times their normal dimensions. They contain many coalescing yellow tubercles. The ventral (anterior) mediastinal glands are similarly enlarged and the cut surface shows a uniformly cheesy parenchyma.

Minute grayish points under the capsule of the liver.

In the spleen, all Malpighian bodies converted into tubercles with yellow opaque centre. In left kidney several minute grayish tubercles.

Of this case, sections of the left lung, left costal pleura, the large caudal mediastinal gland and the spleen were examined.

The tubercles from the lungs do not differ from those found in spontaneous tuberculosis. The centre is as a rule necrotic and contains a few tubercle bacilli. Outside of this is a zone of nuclear fragments and entire nuclei, shading off into a peripheral zone of epithelioid and lymphoid cells and cells with spindle-shaped nuclei. Giant cells are present in nearly all the tubercles. The new growth on the left side of the thorax consists essentially of newly formed vascular fringes, in which are imbedded minute tubercles with scarce bacilli and occasional giant cells.

In the caudal mediastinal gland the tubercles vary from a mere giant cell to those 2 mm. in diameter and centrally necrosed. The latter are like those in the lung tissue. The bacilli in some necrotic foci are numerous, in others few in number.

In the spleen the follicles contain epithelioid cell foci, some of which are centrally necrosed and which contain many bacilli.

No. 88 (Bovine culture IV). Red and white cow. Teeth very much worn, probably 12 years old, dehorned. Not pregnant.

No deposit in subcutis at point of inoculation. The eruptions of tubercles on the costal pleura of the right and the left side are in character very much like those of the preceding case (No. 63), but less extensive.

Right lung. Lobes adherent to pericardium. Adhesions readily severed. On convex surface of the caudal lobe of this side a considerable number of flattish sessile tubercles from $\frac{1}{16}$ to ½ inch broad. The caudal aspect of this lobe is similarly beset with them, but in less abundance. Along the margin of this lobe are loosely attached, small, elongated masses of tubercles. At the same situation in this lobe as in the sputum cases, there is a fluctuating tumor, about two inches in diameter, slightly projecting. It consists of a capsule with nearly smooth walls enclosing a soft caseous mass. It is surrounded by a zone of small necrotic tubercles and with lobules containing numerous minute, grayish foci.

On the cephalic lobe of the right lung are a considerable number of grayish tubercles. Throughout all lobes are scattering tubercles in the lung tissue, some very minute, others larger and opaque, yellowish in color.

The pleura in the dorsal mediastinal space is beset with a large number of small tubercles; similarly the pericardium. The right half of the diaphragm is beset with flattened aggregations of tubercles. Between the ventral lobe of the right lung and the pericardium, and fastening them together, is a mass of newly formed connective tissue and fat enclosing numerous softened foci.

The large dorsal mediastinal lymph gland is enlarged and contains a large number of yellow tubercles. The central portion of the gland is uniformly caseous. In the left bronchial gland a small number of tubercles; in the ventral mediastinal glands a considerable number. Organs of the abdomen appear free from tuberculous changes. Of this case only the tubercles disseminated through the lungs were examined microscopically. For this purpose the left lung was chosen. The tubercles are small, compact up to 1 mm. in diameter. In structure they are indistinguishable from the spontaneous bovine tubercles. The bacilli in them are few in number, rather short, thick and deeply stained.

A summary of the outcome of this last experiment may now be made. The points of difference between the inoculation disease produced by bovine and by human (sputum) bacilli are several.

1. The bovine cases either remained stationary in weight or lost slightly, while the sputum cases gained 75 to 85 pounds. Still, the age of one of the bovine cases may be partly responsible for stationary weight.

2. There was marked fever in the bovine cases for three weeks after the inoculation; practically none in the sputum cases.

3. There were well-marked differences in the lesions produced. In the sputum cases the lesions are nearly the same and consist in:

(*a*) A tumor in the right caudal lobe of the lung, about 1 inch in diameter, projecting somewhat above the surface of the lung. This represents the place where the needle penetrated into the lung tissue and deposited the tubercle bacilli. In each case the contents of this tumor were softened and converted into a curdy mass, enclosed in a thin-walled capsule, smooth internally. The disease was not spreading from this point nor were tubercles visible in the lymph glands of the lungs and thorax, excepting in one gland of No. 39 (Sputum V).

(*b*) The free margin of the right lung and the pleural covering of the ribs on the right side were beset with a new formation of loose vascular fringes or shreds in which in only one case some minute nodules could be felt, and some flattish, pediculated masses, which did not resemble tubercles at all histologically, or which were only abortive forms of the same.

Among the bovine cases we have the following characteristic points to note:

(*a*) Disseminated tuberculosis of the lungs, severest and fatal in No. 71, the youngest; least pronounced in the oldest, No. 88. Associated with this a local disintegrated focus in the lungs of No. 88.

(*b*) Tubercular deposits on lungs, pericardium, diaphragm and the ribs, resembling closely the product of the natural disease in cattle. Extension of the eruption to the costal pleura of the left side.

(*c*) Extensive tuberculosis of all or nearly all the lymph glands of the thorax, including both mediastinal chains.

(*d*) Slight tuberculosis of other organs, spleen, liver, kidney, in two out of three cases.

A summary of the three separate tests on cattle (including the published experiment), in which twelve animals were used, shows that 6 animals were inoculated with human bacilli, 5 with bovine bacilli; 1 animal was inoculated with swine bacilli.

Of the sputum cases, 1 showed no disease; 2 showed very slight lesions; 3 showed only local lesions without dissemination.

Of the bovine cases, 2 died of generalized disease; 2 showed extensive lesions; 1 showed less extensive lesions.

In the swine case the lesions were less extensive than in the bovine cases.

TABLE VI.—CATTLE.

Designation of culture.	Total age of culture.		Number of transfer.	Age of culture used.	Amt. of suspension of bacilli injected.	Designation of animal.	Age, etc.	Original weight in pounds.	Final weight in pounds.	Date of inoculation.	Result.
	mos.	days		days							
Bovine I	5	4	3rd	10	4 cc.	No. 284	heifer, 2½ yrs. old, common stock, pregnant.	(?)	(?)	May 4, 1895	Died in thirty-five days of generalized tuberculosis.
Nasua narica	9	9	8th	10	4 cc.	No. 300	heifer, 2½ yrs. old.	(?)	(?)	May 4, 1895	Killed June 27, 1895, no lesions.
Sputum II	5	9	8th	9	2 cc.	No. 2616	yearling.	520	580	May 1, 1897	Killed July 1, 1897, lesions very slight.
Sputum III	2	15	4th	9	2 cc.	No. 2634	yearling.	410	480	May 1, 1897	Killed July 1, 1897, lesions very slight.
Bovine II	5	2	5th	9	2 cc.	No. 2635	heifer, 2½ yrs. old.	650	710	May 1, 1897	Killed July 1, 1897, extensive pearly disease in thorax and abdomen.
Swine I	11	23	16th	13	2 cc.	No. 2672	heifer, 2 years old.	620	660	May 1, 1897	Killed July 1, 1897, well-marked pleural tuberculosis with invasion of lymph glands.
Sputum IV	6	10	7th	9	2 cc.	No. 79	yearling, about 1½ years old.	525	610	Aug. 26, 1897	Killed Oct. 27, 1897, abscess in lungs at point of injection, new vascular tissue on ribs and lungs.
Sputum V	1	19	3rd	9	2 cc.	No. 39	cow, 4 years old.	675	750	Aug. 26, 1897	Killed Oct. 27, 1897, lesions same as preceding.
Sputum VI	1	12	3rd	9	2 cc.	No. 76	cow, 3 years old.	865	950	Aug. 26, 1897	Killed Oct. 27, 1897, lesions same as preceding.
Bovine III	4	7	5th	9	2 cc.	No. 71	bull, yearling.	645	575*	Aug. 26, 1897	Died Sept. 12, 1897, disseminated tuberculosis of lungs, liver.
Bovine IV	4	3	5th	9	2 cc.	No. 88	cow, about 12 years old.	850	850	Aug. 26, 1897	Killed Oct. 27, 1897, many minute tubercles in lungs, tuberculous deposits on pleura.
Bovine V	4	3	5th	9	2 cc.	No. 68	cow, about 6 years old.	875	870	Aug. 26, 1897	Killed Oct. 27, 1897, disseminated tuberculosis of lungs and spleen.

* Two days before death.

ON THE SIGNIFICANCE OF VARIETIES AMONG TUBERCLE BACILLI.

The foregoing experiments, while they show unmistakably the close relationship existing among the various cultures studied, nevertheless justify us, if only to guide and stimulate further study, in establishing a distinctively human or sputum and a bovine variety of the tubercle bacillus. It might be better to omit the host designation of such varieties, in order to anticipate assumptions that they are necessarily limited to the host whose name they bear. Still, the convenience of using the host's name is so great that I shall succumb to it. The characters upon which the bovine variety may be based reside, morphologically, in the invariably short, straight form and in the greater resistance of this form to modifying influences of culture-media; biologically, in a greater resistance to artificial cultivation and in a much greater pathogenic activity towards rabbits, guinea-pigs and cattle.

There is proof, furthermore, of the existence of slightly varying characters even within the varieties proposed. Among the bovine forms studied, slight variations in virulence were noticeable. Among the sputum forms, variations in size, in capacity for cultivation, and in pathogenic activity have been observed. The differences between Sputum culture I, on the one hand, and Sputum cultures II to VII, on the other, are quite pronounced. In spite of these variations, mammalian tubercle bacilli may still be considered as forming a fairly compact group when compared with the tubercle bacilli of birds, which are but slightly virulent towards the guinea-pig, so susceptible to the mammalian type.*

In regard to the bovine and the sputum varieties, I have, in a former paper, pointed out certain differences of behavior in the body which may be of considerable significance in explaining their divergent characters. In an organism like the human body, manifesting a certain amount of resistance to the tubercle bacillus, the sputum type multiplies only to a limited extent, unless it can produce dead tissue or gain an exit upon mucous membranes secreting pathological products, where it seems to flourish as a saprophyte. Similar conditions are now and then observed in animals inoculated with human bacilli. Immense numbers of bacilli are occasionally found in necrotic foci, but very few in the surrounding zone of living tissue. In the many sections examined recently, the necrotic foci were the ones in which human bacilli were to be found, if detected at all. The bovine type differs from the former in a far less saprophytic growth. In the pathological secretions and in the caseous masses the bacilli are relatively scarce. This difference may be a result of their adaptation to the bovine body, in which cavities of the lungs and catarrh of the air tubes are far less common. In other words, certain differences in the type of reaction tend in the one case to make the human bacillus more saprophytic, the bovine more parasitic.

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* I have, unfortunately, been unable to study avian bacilli in connection with the series of this article, because opportunities for making cultures from fowls were wanting. The study of cultures of unknown or indefinite history I have not regarded as likely to yield trustworthy evidence.

Coming to the more practical aspect of this subject, we may present, in view of the relationship between the bovine and the sputum variety of the tubercle bacillus, two propositions for discussion:

1. The sputum bacillus is incapable of finding a foothold in the bovine body.

2. The bovine bacillus may pass to the human subject, owing to its higher pathogenic power.

The transmission of human tuberculosis to cattle has been experimentally essayed by Bollinger, Crookshank, Baumgarten, Sidney Martin, the writer and Frothingham.

Bollinger * reported in 1894 an experiment performed in 1879. A calf three months old was inoculated into the peritoneal cavity with fluid from a tuberculous human lung. The calf was killed in 7 months. On the mesentery and the peritoneal covering of the spleen there were found pediculated tumors from the size of a pea to that of a walnut. The microscopic appearance corresponded completely with that of the spontaneous pearly disease of cattle. The retroperitoneal and mesenteric glands were tuberculous. All others were normal. The little drawing which Bollinger published with the article gives one the impression of smooth, roundish tumors similar to those I have described as resulting from the injection of Sputum cultures IV and VI. They have not the

* *Münchener med. Wochenschr.*, 1894, No. 5.

nodulated appearance presented by the spontaneous eruptions or those produced by bovine cultures.

Crookshank * injected sputum containing numerous tubercle bacilli into the peritoneal cavity of a calf. The calf died after a prolonged illness on the 42nd day.

> Extensive lesions were discovered at the post-mortem examination. The mesentery was adherent to the rumen; the liver was adherent to the diaphragm. There was extensive tubercular deposit at the seat of inoculation and an abscess the size of a walnut. Extending over the mesentery from this point there were hundreds of wartlike, fleshy new growths, some quite irregular in form, others spherical or button-shaped. There were similar deposits on the under surface of the liver, on the spleen, in the gastro-splenic omentum and on the peritoneal surface of the diaphragm. The spleen was adherent to the rumen, and on dissecting away the adhesions, another abscess was opened. On the under surface of the liver was a third abscess about the size of one's fist, which burrowed in the depth of the liver substance.
>
> On microscopic examination, extremely minute tubercles were found disseminated throughout the lungs and liver. Tubercle bacilli were found in these organs and in the peritoneal deposits. The pus from the liver abscess contained streptococci. The calf died of pyæmia, a result to be anticipated if sputum be employed for inoculation, but sufficient time had elapsed for pronounced local infection leading to acute miliary tuberculosis.

I quote this experiment more fully so that it may discredit itself as a means of deciding whether sputum can produce general miliary tuberculosis in cattle. We know as yet too little of the influence of concurrent infections to place any reliance upon the outcome of this experiment as a demonstration of the transmissibility of human bacilli to cattle.

Baumgarten † briefly mentions an experiment in which human bacilli in cultures had but little local effect after intraocular inoculation upon a calf. Material from a bovine pearly nodule produced typical local and general miliary tuberculosis in another calf.

Sidney Martin ‡ fed one kilogram of tuberculous material from a cow to 4 calves at one meal with their food. Within 4 weeks tubercles were found in the Peyer's patches, mesenteric and bronchial glands and in the lungs of one animal. After 8 weeks, another calf presented the same lesions plus tuberculosis of the posterior mediastinal and the cer-

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* *Trans. Pathol. Soc.*, London, 1891, 332.

† *Jahresbericht* for 1891, footnote, 666.

‡ Report of the Royal Commission on Tuberculosis, 1895. Appendix, p. 18.

vical glands. The third calf, killed after 12 weeks, showed ulceration of the small intestine and cæcum and lesions of the pleura, besides those mentioned above. The fourth calf, killed after 37 weeks, had tuberculosis of the pleura in addition to that of the various lymph glands already mentioned.

Four calves were fed at one meal with 70 cc. of sputum containing a large number of tubercle bacilli. Three calves, killed after 4, 8 and 12 weeks respectively, had 53, 63 and 13 nodules respectively in the small intestines, chiefly in Peyer's patches. In the fourth calf, killed after 33 weeks, no lesions were found.

Two calves received at one feeding 440 cc. of sputum, containing a large number of tubercle bacilli. In one calf, killed in 8 weeks, 13 tuberculous nodules were found in the intestine. The mesenteric glands were also affected. The other calf, killed in 19 weeks, was found without any lesions.

The author infers from these experiments that "in the case of tuberculous sputum we are dealing with material which is less infective to calves than bovine tuberculous material."

Frothingham * carried out some experiments of a similar character for the Mass. Cattle Commission. A culture of human bacilli was used, isolated from the liver of a child and about 1 year old. Two calves, 3 months and 3 weeks old respectively, received suspensions of the culture into the peritoneal cavity. In both only slight local nodules were produced, some resembling spontaneous tubercle, others tending towards granulation tissue. Two calves, 3 weeks and 2 months old respectively, were inoculated into the trachea. In one case the large local abscess in the muscles of the neck indicated a deposit there of much of the material destined for the lungs. In the liver and lungs a small number of minute tubercles, devoid of tubercle bacilli, were found. In the other calf lesions were absent. Thus, in spite of the immature age of these animals, the tubercle bacillus may be said to have had but a trifling local effect on them. The tests on guinea-pigs indicate a very attenuated culture. Three additional calves inoculated subcutaneously with sputum containing many tubercle bacilli showed lesions equally slight.

The defect of most of these tests is the absence of parallel experiments with bovine tubercle bacilli. Inasmuch as we do not yet know anything concerning the relative resistance of cattle to tuber-

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* Report of Mass. Cattle Commission for 1897. See also *Zeitschr. f. Thiermed.*, 1897, 330.

culosis at different periods of life, nor the relative effect of cultivation on the bacilli and of different modes of inoculation, such parallel experiments are quite essential. Of these few experiments, Bollinger's is the only one which might be regarded as favoring the susceptibility of very young cattle to human bacilli. Even here the disease penetrated only to the mesenteric and retroperitoneal lymph glands.

A summary of the inoculation experiments made by the writer upon cattle have already been given. Here we need simply to report the most salient features. These are—

1. The purely local, restricted character of the lesions in cattle due to sputum bacilli. Lesions were not found beyond the place of deposit or in the lymphatic glands to which these are tributary, excepting in one animal (Sputum V culture). In this case we find a multiple tubercular focus measuring *in toto* about 1 to 1.5 cm. in one of the dorsal mediastinal glands. This is the only affected gland found in the 5 cases. Considering the fact that the bacillus was one of the more attenuated, that on the other hand the animal was fully 4 years old, I am strongly inclined to look upon this isolated instance of lymph gland infection as a latent infection with bovine bacilli which was probably favored by the depressing effects of the injection into a slight activity. I take this ground because of its isolated occurrence and the very typical bovine character of the lesions. It is not unusual for such a slight lesion to be encountered in animals which have failed to react towards tuberculin, for experimenters agree upon a margin of error of 5 to 10 per cent, the lesions found in such cases being usually quite insignificant. Had the culture used not shown a virulence not only not above but slightly below the average virulence of the other sputum bacilli, as tested on a rabbit and on guinea-pigs, the above inference could not be justly drawn.

2. The atypical tissue changes induced by the sputum bacilli which tended chiefly towards granulation tissue. Tubercle formation recognizable as such was uncommon. Besides the lymphatic focus cited above as of doubtful origin, tubercles approaching nearest the bovine type were encountered in the primary lung focus in the youngest animal (Sputum IV).

Some writers, including Bollinger and Baumgarten, are inclined to regard the pediculated masses produced on serous membranes by the inoculation of sputum or of cultures (Sputum IV, VI) as something specific and as deciding positively the transmissibility of human bacilli to cattle. Leaving aside the abortive, atypical, microscopic structure of the pediculated masses produced in the writer's series, and assuming that the inoculation of young calves may furnish lesions of a more typical character, we may present anatomical and histological facts which go far to show that there is nothing characteristic or peculiar about them or about pearly disease in general.

On the omentum and the pleura of cattle not tuberculous there may exist in many cases patches of delicate filamentous fringes, probably in all cases the result of some local inflammation. These fringes are microscopic in size and only visible to the naked eye when they are present in large numbers and congested. My attention was first called to them in the study of Texas fever. Owing to the congestion of the whole portal system, these fringes, massed together, appeared as red patches on the omentum. The microscope showed them to consist of a capillary encased in a sheath of delicate fibrillar tissue and the latter covered by the endothelium of the peritoneum. Recently I have demonstrated the same fringes in slightly raised, reddened patches of the costal pleura in otherwise healthy cattle. Here they are quite inconspicuous, but may be demonstrated by seizing the surface of the membrane with delicate forceps. Invisible threads hold the latter near the membrane. In the fresh condition their structure is like that of the fringes on the omentum. In some I was unable to demonstrate a capillary. In all cases they consist of the wavy fibrillar tissue devoid of nuclei, but containing at times a few granule cells, recognized as acidophile leucocytes in stained sections. In sections, these fringes could be traced from their attachment to the serosa. The fibrillar tissue is directly continuous with the subserous layer of like tissue and the endothelial layer of the pleura is continuous over these fringes. The immediate union of fringes containing a capillary with the pleura was not demonstrated in the limited number of sections examined. Besides these of the simplest structure, others are seen in an active state of proliferation and infiltrated with lymphoid and acidophile leucocytes.

These fringes or filaments, probably the result of traumatic and other inflammation, form an excellent place of attachment for the injected tubercle bacilli. Here processes of epithelioid and giant-cell formation go on rapidly when bovine bacilli are present, whereas the sputum bacilli tend to lead only to granulation tissue, with slight abortive tubercle formation. These new formations, subject to pressure and friction, may undergo hæmorrhage, anæmic necrosis due to twisting of the pedicle, and subsequent increase of the connective tissue stroma around the dead fringe. In this way those peculiar smooth nodes probably arise which are briefly described under the cases inoculated with Sputum cultures IV and VI. It is, furthermore, evident from the inoculation experiments that, among other causes, the tubercle bacillus itself is one inducing the formation of granulation tissue. The highly vascular, velvety layers of granulation tissue carpeting the costal pleura in most of the cattle inoculated is a sufficient proof of the existence of this tendency. The pearly disease may thus be a product of at least two factors, the presence of this tissue as a result of former proliferation, and the inherent tendency of the serous membranes of cattle to respond by the formation of these vascular fringes to tubercles situated under the pleura and to bacilli discharged subsequently from such tubercles. But still other factors may be concerned.

That a certain retarded movement of the disease process may produce pearly disease in other animals has been answered in the affirmative for rabbits by Troje and Tangl.* These authors inoculated subcutaneously bacilli from cultures and material from the tuberculous lungs of rabbits which had been intimately mixed with iodoform and kept in the dark in contact with it for one or two weeks. In two rabbits, from two separate experiments, chloroformed respectively 7 and 9 months after inoculation, pediculated tubercles of the serous membranes were found. Those not containing angular concrements, and therefore sectionable, were peculiar in the possession of a very large number of very large giant cells.

These observations I am able to confirm by one case, the rabbit inoculated with the culture of Sputum VI. This rabbit was chloroformed 3½ months after the intravenous injection of tubercle bacilli. Its weight had risen from 1305 to 2061 grms. The notes on this case are briefly as follows:

Organs apparently unaffected (brain and cord not examined), excepting the liver, kidney and lungs. The liver contains a small number of 1 to 2 mm. centrally caseous foci. The kidneys show a considerable number of grayish opaque 1 mm. tubercles. The lungs collapse well. They are everywhere beset with translucent bodies, seen with some difficulty, owing to their glassy appearance. Some have a minute, point-like, opaque centre. In the small cephalic lobes there are larger confluent patches, especially on the margins, of a peculiar grayish-translucent appearance. Sections of hardened tissue show the distinctly epithelioid character of the tubercular changes, with very few giant cells. Necrosis only in the largest foci. Tubercle bacilli fairly abundant in the necrosed centres, elsewhere scarce. From the marginal tubercles finger-like prolongations, precisely like those of the bovine pleura, project into the pleural cavity. They consist mainly of rapidly proliferating connective-tissue cells imbedded in a very delicately fibrillar meshwork. The basal portion of the fringe is partly covered with endothelium continuous with that of the pleura and contains a number of capillaries in transverse section. Similar vascular fringes appear on the opposite margin of the same section.

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* *Deutsche med. Wochenschrift*, 1892, 191.

It is not improbable that if this animal had been allowed to live as long as those of Troje and Tangl, tubercle bacilli would have found their way into these fringes and produced the so-called pearly disease. This rabbit was allowed to live longer than any other of the sputum series. We may thus assume that neoplasms of the pleura in rabbits may be looked for after the third month. These observations go far to show that the eruption of tubercles on the pleura is not so much an expression of peculiarity on the part of the tubercle bacillus as it is an expression of a certain grade of virulence of the bacillus in an organism possessing a certain grade of resistance to it. To these conditions may possibly be added a pronounced tendency in certain animals towards production of granulation tissue of a certain type on serous membranes.

Troje * has also described a rare case of pearly disease in man, affecting the pleura. Here, possibly, the previous existence of outgrowths like the fringes on the serous membranes of cattle may have been responsible for the peculiar localization of the tubercle bacillus.

Putting all the facts obtained by experiments upon cattle together, it would seem as though the sputum bacillus cannot gain lodgment in cattle through the ordinary channels. These avenues, well provided with protective mechanisms, receive the bacilli probably one at a time. However closely the sputum and the bovine bacillus may be related, it seems as if under ordinary circumstances the former would fall an easy prey to destruction. This inference will gain in weight if we bear in mind that the far more potent bovine bacillus produces in at

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* Loc. cit.

least 50 per cent of the spontaneously infected cattle a purely local disease, which probably would remain so if the animal were surrounded with favorable conditions.

The second and most important proposition, the transmission of bovine bacilli to the human subject, has been much discussed in recent years, without, however, bringing us any nearer to definite knowledge.

A recent compilation of cases of presumable transmission from cattle to man, both through wounds of the skin and through the digestive tract in milk, by Ravenel,* gives perhaps the most condensed account up to date. To these may be added a most interesting case reported by Coppez.† A girl 17 years old had a wound on the palmar aspect of the third finger between the 2nd and 3rd phalanx, which became infected with tubercle bacilli during milking. The original lesions gave rise, within 6 months, to over 35 subcutaneous abscesses situated in different parts of the body. There were two on the right hand, four on the elbow, two on the shoulder-blade, several on the right cheek, two on the left palm, one on the back of the neck, a dozen on the buttocks and thighs, four on the left leg, three on the sole of one foot and one on the big toe. Most of these were curetted, and iodoform applied 6 months after the beginning of the disease. Subsequently more appeared, in all from 60 to 66. The author describes the appearance of these foci as follows:

At a certain point, always in the neighborhood of a pre-existing focus, a thickening of the subcutaneous tissue appeared, associated with increased heat of the skin and pain on pressure. The heat soon disappeared, and a fluctuating livid tumor opened itself and discharged pus and later a yellowish serum. These various abscesses healed slowly, and within a year all had disappeared. At no time could any visceral lesions be recognized, but one eye became involved. The nature of the affection was demonstrated by inserting some pus from an abscess into the eye of a rabbit. There was but slight reaction at first. Subsequently the whole eye became diseased, the lymph nodes of the neck being greatly enlarged. After several months death ensued.‡

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* *Journal of Compar. Medicine and Vet. Archives*, December, 1897.

† Un cas de tuberculose cutanée et oculaire sans manifestations viscérales, *Rev. gén. d'ophthalm.*, xv (1896), 433.

‡ In an article written just before the discovery of the tubercle bacillus, Creighton (Bovine tuberculosis in man, *Journ. Anat. and Physiology*, xv (1880), 1-177) describes 12 cases, following one another quite closely in the same hospital, which he considers to be afflicted with the bovine disease. This type of tuberculosis was characterized by eruptions on the serous mem-

If bovine bacilli may invade the human body without let or hindrance, we have not only food infection through milk and milk products to guard against, but also the inhalation disease to which men are exposed in stables containing tuberculous cattle. What proportion of tuberculous subjects may derive their infection from these sources we do not know. Now that we have established some fairly pronounced differences between bovine and sputum bacilli, the whole discussion might be cut short by the suggestion that the time has come to stop citing old and doubtful cases and to go to work to study with care the tubercle bacilli from cases of supposed animal origin, so that some experimental, trustworthy basis may be formed upon which to found statistics. While this is in truth what will have to be done and is the goal which has been aimed at from the outset in this tedious work, it will take much time and persistent attention to collect evidence of this kind. In the meantime the relation of bovine to human tuberculosis must be somehow defined before a fairly helpless and frightened public. It seems to me that, accepting the clinical evidence on hand, bovine tuberculosis may be transmitted to children when the body is overpowered by large numbers of bacilli, as in udder tuberculosis, or when certain unknown favorable conditions exist.* To prevent this from occurring, a rigid, periodic dairy inspection and the removal of all suspicious udder affections and all emaciated animals is as much as public health authorities can at present demand. Any measures beyond these belong to agriculture, with which the public health has no business to meddle, without endangering the chances of

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branes, large, grayish-white medullary tumors in the lungs, frequently assuming the form of a wedge, and by certain microscopic peculiarities. These cases may have been due to some other organism. Quite recently Askanazy (Ueber tumorartiges Auftreten der Tuberkulose, *Zeitschr. f. klin. Med.*, xxxii (1897), 360), besides giving a good bibliography, cites 2 cases in full in which the peculiar type of disease leads him to suggest their relation to pearly disease in cattle. These articles simply impress upon the reader that the true interpretation of such cases will remain obscure until the etiological agent has been studied. The variable character of the tissue reaction to the tubercle bacillus, according to the species, deprives the pathological study, taken alone, of any decisive value.

* Probably much the same as in glanders. The number of glandered horses in certain places is out of all proportion to the cases in men who are continually handling and cleaning them.

gaining authority to enforce its own necessary measures. If the evidence gained by pathology in the future should reveal a greater danger than is here assumed, the scientific basis of such evidence will, I think, force all additional measures needed.

But for the student of etiology the problem does not end in the differentiation of varieties. It reaches out much farther than this and involves some puzzling questions. The most important one bears on the possible changes which the tubercle bacillus may undergo during its prolonged sojourn in the human body. I have already referred to one phase of this question in mentioning the saprophytic growth of the sputum bacillus in the affected lungs and necrotic tissue, as contrasted with its slight multiplication in living tissue and with the generally slight multiplication of the bovine bacillus in the tissue of cattle. This question is a very complicated one, and nothing is easier than to reason in a circle about it, because of the entire absence of data. The first hypothesis to be considered is that which assumes the conversion of the bovine bacillus into the sputum bacillus in the human body. This hypothesis would deny us all possibility of utilizing different characters of tubercle bacilli in tracing their source. If the bovine variety may enter into the digestive tract as such, and after a more or less prolonged interval emerge from the secondarily affected lungs as the sputum variety, we may as well give up all further study of the tubercle bacillus. The accumulated evidence of bacteriology is opposed to this view. Nevertheless, it has been unwittingly assumed by Klebs, in considering intestinal infection with the bovine bacillus, as a common cause of tuberculosis.* As a support of the view that tubercle bacilli change but very slowly, I may again mention the inoculation test upon a rabbit of Bovine culture I. About three years after the first test, the second test resulted in only a very slight prolongation of the disease. The first rabbit succumbed in 17, the second in 22 days. It was thus after 3 years of artificial growth still far above sputum bacilli in pathogenic power.

The question of phthisis as secondary to infection by way of the digestive organs is, however, one needing more attention, for experimental results in this direction are quite suggestive. In all mammals the lungs are evidently the most favored place of tubercle bacilli, and wherever the latter may be deposited, they sooner or later, unless the disease is checked, reach that organ, where the process spreads more rapidly than elsewhere. This march from the place of infection is not infrequently partially concealed by reparative processes. A recent case in point is that of a guinea-pig inoculated with a culture of Sputum VI:

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* Die kausale Behandlung der Tuberkulose, 1894, p. 34.

Aug. 29, 1897. Male guinea-pig, weighs 420 grams. Receives subcutaneously in the flank 0.5 cc. of a clouded suspension of tubercle bacilli (Sputum VI) 9 days old.

Nov. 8. Breathing now labored; has been active since inoculation.

Nov. 11. Respiration labored and almost convulsive; hence chloroformed; weight 431 grams.

No ulcer at point of inoculation.

Kneefold glands of this side as large as peas, yellowish in color, consisting of a thin-walled sac and homogeneous contents, resembling thick cream. Kneefold glands of opposite side moderately enlarged, but without necrotic changes. The pelvic, renal, retrogastric and mesenteric glands enlarged slightly, but without necroses. Spleen small, shows grayish specks disseminated through it. Liver and kidneys appear normal. Two small tubercles on left testicle.

Lungs do not collapse. They feel firm everywhere. On section most of the lung tissue is consolidated, of a grayish-white color. No fluid expressible from air tubes. In sections of the lungs, the infiltration consists chiefly of epithelioid cells with smaller, more deeply stained nuclei intermingled or in patches. No necrosis. The liver was, through an oversight, not preserved. The spleen shows in each follicle a rarefied area occupied by epithelioid cells and a few tubercle bacilli. No necrosis.

In this susceptible animal the only lesions threatening life or even conspicuous were those in the lungs. Those in the spleen were slight, possibly stationary. The disintegrated superficial glands might have discharged outwardly later on and healed. This is but one of a number of cases I have observed in the past 10 years in which there was progression in the lungs with almost complete destruction of the organ and retrogression elsewhere, showing itself in the liver by shrinking and furrowing of the organ.*

With the two facts before us that tubercle bacilli gravitate, as it were, towards the lungs in all the susceptible mammals, and that they may conceal their movements in the body quite effectually, we must regard infection through the digestive tract as a source of phthisis at least deserving more attention. The only question to interest us here is the relation of the bovine bacillus to this process. Only much painstaking work will enable us to learn whether the human body can produce such a great modification of the bovine bacillus or not.

Another more acceptable hypothesis concerning changes in the tubercle bacillus would assume a slow, continuous modification which in the past has developed the sputum variety on the one hand and the bovine variety on the other. The future may enable us to establish not only other varieties linked to certain animal species, but also varieties which produce slightly different forms of disease in man himself.† The saprophytic life of the sputum bacillus in phthisis may be the cause of a very slow downward descent of its invasive power, which in the course of one or more generations may become perceptible both to the bacteriologist and to the clinician, and express itself to the statistician in the gradually falling curve of tuberculosis, as shown by the vital statistics of the past 40 years.‡

The outcome of such slow changes would lead to the establishment of numerous minor varieties because of the great opportunity which tubercle bacilli have in our congested, abnormal city life of selecting their variously predisposed victims. Thus the more attenuated varieties may find their invasion obstructed in the more vigorous, but not in children or certain predisposed individuals. It is needless for me to illustrate these possibilities still farther. They have their parallels in nature all about us. Only one practical suggestion may be made, in view of the probable discovery of minor varieties of human bacilli. Tuberculous patients should not be indiscriminately exposed to other tuberculous patients simply because both are affected with a disease which bears the same name.*

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* This generalization has been recently emphasized by Spengler (*Ztschr. f. Hygiene*, xxvi (1897), 321) as follows: The retarded course of an inoculation tuberculosis expresses itself without exception in a (greater) prominence of the lung disease with simultaneous retardation of the liver and spleen affection.

† Recently I have been able to study cultures of tubercle bacilli from a rapidly progressive tuberculous gland of the neck in an adult. These bacilli are slightly less virulent than the sputum bacilli described.

‡ S. W. Abbott. The Vital Statistics of Massachusetts. Rep. of the State Board of Health for 1896, p. 787-8.

A more exhaustive experimental study of the tubercle bacillus away from the many variable factors acting upon the human body, which will lead to a better standardizing of virulence or invasive power, may help these speculations to find some substantial basis. That much is still to be learned concerning the etiology is evident from the want of unanimity as regards the channels of infection. The views of Koch and his followers of the transmission of the infection in dust and spray would tend to favor the hypothesis of gradual attenuation and of the production of minor varieties. Baumgarten's hypothesis of the transmission of the tubercle bacillus from parent to child before birth implies the transmission of tubercle bacilli either in a continual struggle with living tissue or else remaining in situations where the bacilli cannot multiply, and hence where a tendency to loss of invasive power by saprophytic growth is impossible. The occasional entrance of bovine bacilli into the human body might open the way for the introduction of a virus of a higher level, provided opportunity for subsequent transmission be afforded.

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* A beginning in this comparative study of different clinical types of tuberculosis has been made by Auclair (*Arch. de méd. exp.* (1897), ix, 1124). This article, which came to my notice after the completion of this manuscript, describes 4 cultures, one from a rapidly progressive tuberculosis of a gland of the neck, one from a slow phthisis, one from an acute meningitis and one from true scrofula. On guinea-pigs the intra-abdominal inoculation of these cultures had nearly the same effect. Hence the author did not hesitate to regard them as identical in pathogenic power, in opposition to the views of Arloing, who considers the bacilli of phthisis and those of the surgical forms of tuberculosis of different virulence. Auclair did not inoculate rabbits, in spite of the fact that Arloing regarded them as the best means of differentiating the bacilli of the different forms of tuberculosis. My own results show that if the intra-abdominal inoculation of guinea-pigs is the only test made, practically no differences worth considering would appear between bovine and sputum bacilli, as regards pathogenic power.

If in this brief summary I have presented nothing but problems to be solved and doubts to be entertained, I feel quite confident that the comparative study of tubercle bacilli will lead to some definite understanding on certain important questions, and eventually to more light on the whole subject of tuberculosis from the preventive as well as the therapeutic side.

The main questions proposing themselves to the investigator are:

1. The study of tubercle bacilli from different types of tuberculosis to determine their relation to the sputum bacillus and the bovine bacillus as regards virulence.
2. The study of the bacilli in primary intestinal disease and in all tubercular disease in children in which the source of infection is assumed to be outside of the family and possibly in the milk.

In attacking these problems the investigator should either keep strictly to the methods suggested in this article, if he wishes to use its results as a basis, or else, in all cases in which the bovine bacillus or one of abnormal virulence is suspected, he should study fresh cultures of the sputum bacillus side by side with the other.

THE RELATION OF THE TOXICITY OF DIPHTHERIA TOXIN TO ITS NEUTRALIZING VALUE UPON ANTITOXIN AT DIFFERENT STAGES IN THE GROWTH OF CULTURE.

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Until within a fairly recent time the filtered or sterilized bouillon in which the diphtheria bacillus had grown and produced its "toxin" was supposed to require for its neutralization an amount of antitoxin directly proportional to its toxicity as tested in guinea-pigs. Thus, if from one bouillon culture 10 fatal doses of "toxin" were required to neutralize a certain quantity of antitoxin, it was believed that 10 fatal doses from every culture, without regard to the way in which it had been produced or preserved, would also neutralize the same amount of antitoxin. Upon this belief was founded the Behring-Ehrlich definition of an antitoxin unit.

The results of tests by different experimenters with the same antitoxic serum but with different toxins proved this opinion to be incorrect. Ehrlich * deserves the credit for first clearly perceiving and publishing this. He obtained from various sources twelve toxins and compared their neutralizing value upon antitoxin; these tests gave most interesting and important information. The results in six toxins, which are representative of the twelve, are as follows: (See Table I.)

From the facts set forth in the tables Ehrlich has derived interesting theories, which, if true, would add greatly to our knowledge of toxins and would also have a very direct influence upon the present methods of standardizing antitoxin. He believes that the diphtheria bacilli in

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* Ehrlich, Die Wertbemessung des Diphtherieheilserums und deren theoretische Grundlagen, *Klinisches Jahrbuch*, vi (1897).

TABLE I.—(FROM EHRLICH.)

Toxin specimen number of Ehrlich.	Estimated "minimal" fatal dose for 250-grm. guinea-pigs.	Smallest number of fatal doses of toxic bouillon required to kill a 250-grm guinea-pig within 5 days when mixed with 1 antitoxin unit. "L_+" Ehrlich.	Fatal doses required to "completely neutralize 1 antitoxin unit" as determined by the health of the guinea-pig remaining unaffected. "L_0" Ehrlich.	$L_+ - L_0$	Data upon "toxin" specimen given by Ehrlich.
2.	0.03	42	32	10	*Preserved 2 years.
4.	0.009	39.4	33.4	6	Old, deteriorated from .003 to .009.
7	0.0165	76.3	54.4	22	Fresh toxin, preserved with trikresol.
9.	0.039	123	108	15	A number of fresh cultures grown at 37° C. 4 and 8 days.
10.	0.001	29.2	27.5	1.7	Precipitated from greatly deteriorated "toxin."
12.	0.0025	100	50	50	Tested immediately after its withdrawal.

their growth produce toxin which, so long as it remains chemically unaltered, has a definite poisonous strength with a definite value in neutralizing antitoxin. This neutralization he believes to be a chemical union, in which 200 fatal doses of toxin for a 250-gramme guinea-pig combine with one unit of antitoxin. The toxin is, however, an unstable compound and begins to change almost immediately into substances which are not, at least acutely, poisonous, but which retain their full power to neutralize antitoxin. These substances, according to Ehrlich, fall into three groups. The first has more affinity for combining with the antitoxin than the toxin itself (protoxoids). The second has the same affinity (syntoxoids). The third has less affinity (epitoxoids).

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* For number 9 only does Ehrlich give the duration of the culture growth in the incubator by which the toxin was produced.

According to him, if a mixture of toxoids and toxin is added to antitoxin, the protoxoids first combine with the antitoxin, then the syntoxoids and the toxin combine in equal proportions, so long as the supply lasts, with the amount of antitoxin remaining, or if there is a surplus, with enough to satisfy them; finally, if any antitoxin remains, the epitoxoids unite with it.

If to a mixture, in which all three toxoids as well as toxin have united with antitoxin, some additional toxic culture bouillon be added, the new protoxoids displace first the epitoxoids and then, if free protoxoids remain, the toxin and the syntoxoids from their antitoxin, and thus liberate as well as add free toxin to the solution.

Ehrlich states as an important law that toxin molecules change into toxoid molecules either by three toxin molecules becoming two toxoid and one toxin molecules, or that two toxin molecules change into one toxoid and one toxin molecule.

The practical conclusion which he draws is that from the absolute amount of poison in a given quantity of bouillon alone, nothing can be determined as to its neutralizing value for antitoxin, and that, therefore, the old Behring-Ehrlich method must be greatly modified; namely, that in any bouillon we must detect not only the amount of toxin but also of toxoids before we can judge of its neutralizing value. This he believes he can accurately accomplish.

He gives an interesting theory to explain the production of antitoxin in the blood. This he does upon the supposition that, when absorbed, the toxin combines with a portion of certain selected cells, and that this portion by its union with toxin becomes, at least physiologically, dead. The cell replaces this dead matter with new and similar substance; after several repeated losses and replacements of this substance the cells produce it in excess. This substance, whether originally in the normal cell or reproduced there, and whether remaining in the cell or thrown out into the circulation, is antitoxin.

Ehrlich attempts to separate into its constituent toxoids and toxin each culture bouillon by a formula worked out by him. He believes that his investigations show that the total amount of pure toxin which neutralizes one unit of antitoxin is 200 minimal fatal doses, and that in ordinary culture fluids, which are mixtures of toxoids and toxin, an antitoxin unit neutralizes 200 fatal doses of toxin minus the number of toxin molecules changed into toxoid molecules. Letting A = number of toxin molecules and Z = epitoxoids, then one unit of antitoxin fully neutralized equals (200 — A — Z) toxoid-antitoxin + A toxin-antitoxin + Z epitoxoid-antitoxin. By toxoids are designated both protoxoids and syntoxoids.

To find epitoxoids his first step is to add as much toxin-toxoid mixture to the quantity of the toxic fluid which just neutralized one unit of antitoxin, as shown by the complete health after its injection of the guinea-pig, as will suffice to produce death on the fourth day. This amount will always be found to be above three fatal doses. This apparently excessive amount he believes to be explained by the fact that part of the almost harmless epitoxoids has been displaced by toxin. Ehrlich considers death on the fourth day to be caused by one free minimal fatal dose of toxin.

Let us, as Ehrlich, lump syntoxoids and protoxoids together under the name "toxoids," then suppose we have a toxin composed of 50 toxin molecules + 50 (syntoxoids and protoxoids) toxoids and 100 epitoxoids. To satisfy these 200 molecules of toxin, toxoids and epitoxoids we add one unit of antitoxin. After chemical combination we have 50 toxin-antitoxin and 50 toxoid-antitoxin and 100 epitoxoid-antitoxin. To displace the epitoxoids from the antitoxin we would have to add enough fatal doses to contain 100 molecules of toxin and toxoids, this would be then 50 of toxin and 50 of toxoid. A half molecule more of each would leave a pure toxin molecule, which would, according to Ehrlich, cause the guinea-pig's death in four days.

Ehrlich's formula to find the epitoxoids is:

$$\text{Epitoxoids} = \frac{200^{*}\,(L_{+}^{\dagger} - L_{0}^{\ddagger} - 1^{\S})}{L_{0} + (L_{+} - L_{0} - 1^{\S})} \text{ which with the above toxin would be } = \frac{200 \times 50}{50 + 50} = 100.$$

[*] 200 = number of molecules of toxin or toxoid required according to Ehrlich to neutralize one unit of antitoxin.

[†] L_{+} = fatal doses of toxin required to kill a guinea-pig in four days after having been mixed with one unit of antitoxin.

[‡] L_0 = fatal doses of toxin required to fully neutralize one unit of antitoxin.

[§] 1, though given by Ehrlich, appears to us to be incorrect, for by his own statement one molecule of toxin and toxoid would be added, not 1 of toxin (1 fatal dose) alone. The amount to be subtracted from the difference between L_{+} and L_0 would therefore be, not one, but a fraction of one.

The above summary merely gives an outline of Ehrlich's most interesting article. To become fully acquainted with the reasons for his theories the article itself must be carefully read. He concludes with the statement that his investigations have cleared up the difficulties surrounding the estimation of the amount of antitoxin in any given quantity of serum, and that the formula resulting from his investigations enables us to determine in any selected toxic diphtheria culture fluid the amounts of toxin and toxoid present, so that a unit of antitoxin is no longer a varying quantity, but of an exact and determinable size and one which can at any time be reproduced.

Interest in both his theoretical reasoning and in his practical conclusions led us to subject both to a series of tests which have, we believe, added some interesting facts to those already published by Ehrlich as well as cast doubts on some of his conclusions.

ORIGINAL INVESTIGATIONS.

In the following pages are given the results obtained by testing the toxicity in guinea-pigs and the neutralizing value upon antitoxin of fluid removed from bouillon cultures of different diphtheria bacilli after varying periods of growth.

Five cultures of diphtheria bacilli were used. Two of these were employed for the most important of the experiments and a short description of these may be of value.

One of them, No. 8, was obtained three years ago from a case of mild tonsillar diphtheria. For these three years this special culture has never been passed through animals, but has been transplanted to fresh bouillon every two to three days. It has kept almost intact its original virulence. It kills 250-grm. guinea-pigs in doses of about .005 cc. of a 48-hour bouillon culture in from four to five days, and produces a toxin in from two to five days of such a strength that usually .005 cc. of the filtered bouillon culture kills within five days most guinea-pigs weighing 250 grammes. The toxin produced by this bacillus has been constantly employed, with apparently uniform results in testing antitoxin.

The other culture, "Greenwood," was obtained six months ago from a case of diphtheria occurring in a localized epidemic in a town situated in an isolated mountainous district. This epidemic was remarkable for the frequency with which paralysis appeared in rather mild cases. This peculiarity is noticed in its effects in animals. The other three cultures were obtained one year ago from cases of diphtheria occurring in New York City. These four cultures are of only moderate virulence and have been transplanted to fresh bouillon every few weeks.

The bouillon used for the development of toxin for these experiments, after being prepared from meat in the usual way, was first poured into a single vessel and made neutral to litmus. After adding to each litre of this common stock of neutral bouillon seven cubic centimetres of normal soda solution, the whole was boiled, filtered, poured into 600 ccm. flasks, stoppered with cotton and covered with filter-paper caps. The bouillon contained two per cent of Witte's peptone and filled the flasks one-half full. The bouillon, after thorough sterilization in the flasks, was inoculated from the pellicle of cultures made 48 hours previously. The growth of all cultures was abundant. After the period of growth at 35° C. had lasted the desired time, a measured quantity was withdrawn from each flask by means of sterilized pipettes. The amounts of fluid withdrawn were mingled together and, after the addition of 0.5 per cent of carbolic acid, stored in small sterilized glass bottles, stoppered and placed in the ice chest and there kept until used. As a rule a fresh vial of "toxin" was used for each experiment, as the exposure to the air of the toxin left in the bottle is apt to cause a deterioration in its strength.

The following tables give the actual and comparative toxicity and neutralizing value of "toxin" removed from bouillon cultures of diphtheria bacilli between the 6th hour and 49th day of their growth. Each table gives the details of a single experiment. It was found impossible to rely upon the L_+ and L_0 values unless all the guinea-pigs were inoculated at the same time. Otherwise slight changes in the condition of the animals and of the toxin were found to make the results somewhat inaccurate or at least subject to suspicion.

TABLE II.

GIVING RESULTS OF TEST OF TOXIN PRODUCED BY BACILLUS 8 AT END OF 6 HOURS' GROWTH IN SLIGHTLY ALKALINE 2% PEPTONE BOUILLON.

Toxin tested with one-tenth of an antitoxin unit on account of the slight toxicity of the fluid.

	Weight of guinea-pig.	Amount of toxic bouillon in cc.	Amount of antitoxic serum in cc.	Result in guinea-pig.	
1	278	3.00	*.00012	No deleterious effects.	
2	234	3.20	.00012	No deleterious effects.	
3	237	3.40	.00012	Slight temporary decrease in weight, no induration.	
4	245	3.60	.00012	Slight temporary decrease in weight, no induration.	
5	242	3.80	.00012	Slight temporary decrease in weight, slight induration.	
6	250	4.00	.00012	Considerable induration. Death 8th day.	
7	245	4.40	.00012	Died 6½ days,	
8	248	.3769	†.38	Died 7½ days,	Control tests of toxin to establish the minimal fatal dose for 250-gramme guinea-pigs.
9	265	.3816	.36	Died 6½ days,	
10	249	.44	.44	Died 5½ days,	
11	221	.40	.45	Died 4½ days,	
12	230	.45	.487	Died 3½ days,	
13	240	.5	.51	Died 3½ days,	

The minimal surely fatal dose for every 250-grm. guinea-pig, estimated from results of above tests, equals 0.45 cc. One-tenth of a unit, 0.00012 cc., of serum neutralized 3.40 cc., or 7.55 fatal doses, of toxin sufficiently to protect the health of the guinea-pig.

The "minimal fatal dose" is here defined, so as to be in accord with Ehrlich, as the amount which will kill most guinea-pigs of 250 grms. weight on the fourth day and the remainder that live longer certainly on the fifth day. The amount of toxin given is calculated according to the gramme weight of the pig, since experience has taught that on the average guinea-pigs weighing between 225 and 275 grammes are susceptible to toxin directly proportional to their weight.

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* .00012 cc. of this serum equals one-tenth of an antitoxin unit as standardized by Ehrlich's test serum.

† Equivalent amount of toxin for a 250-grm. guinea-pig.

TABLE III.

GIVING RESULTS OF TEST OF TOXIN PRODUCED BY BACILLUS 8 AT END OF 22 HOURS' GROWTH IN SAME CULTURE AS TESTED AT 6 HOURS.

	Weight of guinea-pig.	Amount toxic bouillon in cc.	Amount serum in cc.	Result in animal.		
				Weight on 5th day.	Amount induration on 5th day.	General condition on 5th day, if alive.
1	260	2.00	.0012	265	None.	Good.
2	257	2.10	.0012	242	Slight.	Good.
3	250	2.16	.0012	237	Considerable.	Good.
4	252	2.20	.0012	215	Considerable.	Sick.
5	265	2.36	.0012	225	Considerable.	Dying.
6	252	2.38	.0012		Marked.	Dead on 5th day.
7	250	2.40	.0012			Dead on 3rd day.
8	248	.016	*.016	Died 6th day.	Control tests of toxin to establish the minimal fatal dose for a 250-grm. guinea-pig.	
9	248	.018	.018	Died 4th day.		
10	232	.0168	.018	Died 6th day.		
11	238	.020	.021	Died 3rd day.		

Minimal fatal dose estimated to be about .019 cc.
$L_0 = 2.06 = 108.4$ minimal fatal doses.
$L_+ = 2.39 = 125.8$ minimal fatal doses.
$L_+ - L_0 = .33 = 17.4$ fatal doses.
Epitoxoids by Ehrlich's formula $\frac{200 \times 16.4}{108.4 + 16.4} = 27.8$.
.0012 cc. of this serum equals one unit of Ehrlich's test serum.

TABLE IV.

GIVING RESULTS OF TEST OF "TOXIN" PRODUCED BY BACILLUS 8 AT END OF 48 HOURS' GROWTH. TESTED vii-6-'98.

	Weight of guinea-pig.	Amount of toxin in cc.	Amount serum in cc.	Result in animal.		
				Weight on 5th day.	Amount of induration on 5th day.	General condition on 5th day.
1	220	.500	.0012	239	Pin-point.	Good.
2	224	.512	.0012	222	Bean-sized.	Good.
3	288	.524	.0012	280	Slight.	Good.
4	257	.536	.0012	248	Moderate.	Good.
5	270	.548	.0012	250	Considerable.	Good.
6	262	.560	.0012	238	Marked.	Good.
7	235	.582	.0012	176	Marked.	Sick (died vii-13).
8	265	.594	.0012	217	Marked.	Sick (died vii-14).
9	250	.604	.0012	192	Marked.	Very sick (died vii-12).
10	280	.616	.0012			Died vii-10 (4th day).
11	264	.628	.0012			Died vii-9 (3rd day).
12	260	.0062	†.0060	Died vii-12 (6th day).		Control tests of toxin to establish the four-day or "minimal fatal" dose for 250-grm. guinea-pig.
13	259	.0067	.0065	Died vii-12 (6th day).		
14	272	.0076	.0070	Died vii-12 (6th day).		
15	246	.0073	.0075	Died vii-10 (4th day).		
16	246	.0083	.0085	Died vii-12 (6th day).		
17	248	.0089	.0090	Died vii-9 (3rd day).		

.0075 estimated minimal fatal dose for 250-grm. guinea-pig.
$L_+ = .610 = 81.33$ fatal doses.
$L_0 = .512 = 68.26$ fatal doses.
$L_+ - L_0 = .098 = 13.07$ fatal doses.
Epitoxoids by Ehrlich's formula 30.'

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* Equivalent of toxin dose for a 250-grm. guinea-pig.

† Equivalent amount for a 250-grm. guinea-pig.

TABLE V.

GIVING RESULTS OF TEST OF TOXIN PRODUCED BY BACILLUS 8 AT END OF 6 DAYS.

Weight of guinea-pig.	Amount of toxin in cc.	Amount of antitoxin in cc.	Result in animal.	
200	.48	.0012	Remained full weight and alive.	
210	.48	.0012	Died with paralysis after 20 days; on 11th well and full weight.	
214	.51	.0012	Died in 3½ days.	
217	.53	.0012	Died in 4½ days.	
212	.0047	*.0055	Died 4th day.	Control tests to establish four-day fatal dose for guinea-pigs.
254	.0056	.0055	Died 7th day.	
208	.0050	.0058	Died 7th day.	
208	.0052	.0062	Died 4th day.	

Estimated minimal fatal dose, .0062.

L_+=.535=86.3 fatal doses.

L_0=.475=76.6 fatal doses.

$L_+ - L_0$=.06=9.7 fatal doses.

Epitoxoids by Ehrlich's formula=20.16.

The toxin from the Greenwood culture could not be tested before 3 days, since up to 48 hours the culture fluid was not toxic. Minimal fatal dose 48 hours = 5 cc.

TABLE VI.

SHOWING RESULTS OF TESTING TOXIN REMOVED AT END OF 72 HOURS' GROWTH FROM GREENWOOD CULTURE.

Weight of guinea-pig.	Amount of toxin in cc.	Amount of serum in cc.	Result in animal.	
220	5.90	.0012	Unaffected.	
260	6.00	.0012	Slight induration.	
248	6.80	.0012	Died 7th day.	
225	7.30	.0012	Died 2nd day.	
228	.050	†.055	Died in 6 days.	Control tests of toxin for minimal fatal dose.
238	.056	.060	Died in 3½ days.	
210	.0546	.065	Died in 3½ days.	

Four-day or minimal fatal dose of toxin estimated at .058.

L_0=about 5.95=102.6 fatal doses.

L_+=about 7.00=120.7 fatal doses.

$L_+ - L_0$=about 1.10=18.1 fatal doses.

Epitoxoids by Ehrlich's formula=29.

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* This culture was developed in a different flask of bouillon from that used for the other tests. The bouillon was from different meat, but was otherwise similar.

† Equivalent for 250-grm. guinea-pig.

TABLE VII.

SHOWING RESULTS OF TESTING TOXIN REMOVED AT END OF 6 DAYS' GROWTH FROM GREENWOOD CULTURE.

Weight of guinea-pig.	Amount of toxin in cc.	Amount of serum in cc.	Result in animal.	
229	.4172	.00012	On 2nd day lost 13 grms., but on 4th day regained 7 grms. and remained well.	
193	.4323	.00012	Died in 50 hours.	
195	.0312	.00400	Died in 2½ days.	Control tests for minimal fatal dose of toxin for 250-grm. guinea-pig.
209	.038	.00452	Died in 3½ days.	

Estimated minimal fatal dose about .038.

One-tenth unit .00012 completely protected guinea-pig from .4172 cc. of toxin = 10.9 fatal doses.

L_+ would equal about 104 fatal doses.

TABLE VIII.

SHOWING RESULTS OF TESTING TOXIN REMOVED FROM SAME FLASK OF GREENWOOD CULTURE AT END OF 7 WEEKS.

Weight of guinea-pig.	Amount of toxin in cc.	Amount of serum in cc.	Result.	
220	2	.0012	Unaffected.	
250	3	.0012	Slight induration, temporary loss in weight.	
217	4	.0012	Temporary induration and loss in weight. Paralysis 20th day. Death 25th day.	
210	5	.0012	Death on 5th day.	
215	6	.0012	Death on 2nd day.	
245	.10	*.1020	Lived, loss 30 grams in weight.	Control tests of toxin to establish the four day "minimal" fatal dose for a 250-grm. guinea-pig.
270	.12	.1110	Lived.	
237	.13	.1390	Lived after recovering from paralysis.	
234	.14	.1495	Died 22nd day, paralysis.	
227	.16	.1760	Died 24th day, paralysis.	
250	.18	.1800	Lived after recovering from paralysis.	
262	.20	.1907	Died 2nd day.	
234	.19	.2030	Died 3rd day.	

Minimal or four-day fatal dose estimated from above tests at .19 cc.

L_+ = about 5.10 = 26.8 fatal doses.

L_0 = " 3.00 = 15.8 " "

$L_+ - L_0$ = about 2.10 = 11.0 fatal doses.

Epitoxoids by Ehrlich's formula, $\frac{200 \times 10}{15.8 + 10} = \frac{2000}{25.8} = 77.5.$

The unusual amount of paralysis in the guinea-pigs receiving much less than the four-day fatal dose is very striking in the above series. Toxin from this culture of shorter duration of growth had this same characteristic, but to a less extent.

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* This column of figures shows equivalent toxin for a 250-grm. guinea-pig.

TABLE IX.

Giving Minimal Fatal Dose; Number of Fatal Doses of Toxin Neutralized by One Unit of Antitoxin Sufficiently to Protect Life of Animal for 4 Days, L_+; to Protect the Health of the Animal, L_0; the Difference between L_+ and L_0; and the Amount of Epitoxoids as determined by Ehrlich's Formula.

Culture 8 development.	Minimal fatal dose in cc.	L_+ in minimal fatal doses.	L_0 in minimal fatal doses.	$L_+ - L_0$ in minimal fatal doses.	Epitoxoids (Ehrlich). Number of molecules in amount of "toxin" required to neutralize one unit.
6 hours	.45	*72			
22 hours	.019	125.8	108.4	17.4	27.8
‡28 hours	.018	91.6	77.7	13.9	28
48 hours	.0075	81.33	68.26	13.07	30
†48 hours	.016	81.2	73.4	7.8	17
† 6 days	.0062	86.3	76.6	9.7	20.16
‡ 3 weeks	.0095	42.3	34.2	8.1	34.3
‡ 8 weeks	.038	31.6	21	10.6	46.8
Culture Greenwood.					
3 days	.058	120.7	102.6	18.1	29
6 days	.038	*104			
7 weeks	.19	26.8	15.8	11	77.5

The above tabulated results obtained from testing the absolute and comparative toxicity and neutralizing value upon antitoxin of fluid removed at different periods in the growth of two cultures are certainly striking, and fully substantiate Ehrlich's claim that the original Behring-Ehrlich definition of an antitoxin unit is very insufficient. The small number of fatal doses of 6-hour "toxin" which .1 unit of

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* Estimated from the number of fatal doses sufficiently neutralized by one-tenth of a unit to protect the health of the guinea-pig.

† The bouillon in which this toxin was formed was different from that in which the others developed.

‡ Tests completed too late to insert tables. Details carried out as in those where full tables have been given.

antitoxin neutralized—7.5, which is the equivalent of about 72 for one unit—surprised us, for, according to Ehrlich's theories, we should expect the "toxin" to be nearer purity then than at 22 hours. It is probable that changes produced in the culture bouillon account for this and later unexpected results. A second peculiar result of the test was the rapid decrease (from 126 to 91) in the number of fatal doses required to neutralize one unit between the 22nd and the 28th hour, to be followed by slight irregular changes (81 at 48 hours, 86 at 6 days) before the final apparently permanent tendency to decrease (42 at 3 weeks, 31 at 6 weeks).

"Toxin" from culture Greenwood in its test at 3, 6 and 49 days shows also very marked variations in the actual and in the comparative toxicity and neutralizing strength of the culture bouillon. We note that at 3 days the culture fluid required 120 fatal doses to neutralize one unit, about the same amount as culture 8 required at 22 hours. The variation in these two cultures is more apparent than real in that, although the neutralizing value of a fatal dose was different at similar periods of growth, nevertheless when we consider that culture 8 began to produce toxin immediately and culture Greenwood only at the end of 48 hours, we see that at about the same period in their toxin production the neutralizing value of a minimal fatal dose was about the same in both.

Before discussing further these tests, let us examine the neutralizing value of a fatal dose in different cultures, grown in similar bouillon, at the same period of growth and of the same culture in bouillon from different sources.

TABLE X.

THE ACTUAL AND COMPARATIVE TOXICITY AND NEUTRALIZING VALUE OF THE FLUID FROM FIVE DIFFERENT CULTURES AFTER SIX DAYS OF GROWTH.

Culture.	Minimal fatal dose for 250-grm. guinea-pig.	Number of fatal doses neutralized by one-tenth of an antitoxin unit.
8	.006	9
213	.085	9.76
236	.030	13.9
238	.043	9.07
Greenwood	.038	10.9

The results given above from the tests of the neutralizing value of the toxin removed from five cultures after 6 days' growth at 36° C., the time when toxin is usually withdrawn by us, reveals a great lack in uniformity in the neutralizing value of a fatal dose, although no such difference exists as in the case of the same culture fluid at widely different periods of development.

The actual and comparative toxicity and neutralizing value of the "toxin" produced by the same bacillus in the fluid removed from two flasks of bouillon derived from different meats but of equal alkalinity is shown by comparison of Tables IV and XI.

The data upon the tests of one culture bouillon removed 48 hours after inoculation with bacillus 8 have been given already in Table IV.

The minimal fatal dose was .0075.

$L_+ = .610$ or 81.33 fatal doses.

$L_0 = .512$ or 68.26 fatal doses.

The data of the test of the "toxin" developed by the same bacillus 8 in a different bouillon is given fully in Table XI, since a comparison of the two is important, in that the uniformity of the two toxins indicates the possibility of the same bacillus under suitable conditions furnishing a toxin of always about the same relative toxicity and neutralizing power.

TABLE XI.

TEST OF TOXIN PRODUCED BY BACILLUS 8 AT THE END OF 48 HOURS IN A BOUILLON DIFFERENT FROM THAT RECORDED IN TABLE IV.

	Weight of guinea-pig.	Amount of toxic bouillon in cc.	Amount of serum in cc.	Result in animal.	
1.	265	1.125	.0012	Unaffected.	
2.	263	1.15	.0012	Slight skin necrosis. No loss in weight.	
3.	270	1.175	.0012	Slight induration. No loss in weight.	
4.	277	1.20	.0012	Death 7th day.	
5.	257	1.30	.0012	Death 4th day.	
6.	250	.011	*.011	Died 6th day.	Control tests of toxin to establish four-day fatal dose in guinea-pigs.
7.	235	.0112	.012	Died 21st day after paralysis.	
8.	250	.0106	.013	Died 6th day.	
9.	205	.0118	.014	Died 6th day.	
10.	264	.0158	.015	Died 5th day.	
11.	240	.0153	.016	Died 4th day.	

Minimal or four-day fatal dose estimated at .016 cc.
$L_+ = 1.30 = 81.2$.
$L_0 = 1.175 = 73.4$.
$L_+ - L_0 = 125 = 7.8$.
Epitoxoids "Ehrlich" = 17.

* The equivalent in a 250-grm. guinea-pig of the amount of toxin given in the actual animal.

Certainly the toxins produced by bacillus 8 in these two different bouillons as given in the Tables IV and XI are remarkably similar in the neutralizing value of a fatal dose, although the toxicity of one toxin as shown in Table XI is only one-half of the other as shown in Table IV. Six toxins developed during the six-days' growth of cultures of No. 8 in slightly alkaline bouillon have at different times in the past three years been used in antitoxin tests, and all have had nearly the same antitoxic neutralizing value for a fatal dose.

These results have encouraged us to hope that, with a closer study of the relations existing between the changes taking place in the bouillon during the different stages of the growth of the bacilli, and of both the development of the toxin and its neutralizing value, we may be able to obtain from any culture of known characteristics at a certain period of its toxin production a toxin of always about the same neutralizing power. A comparison between different laboratories of the neutralizing value of the toxins produced by their bacilli would help to obtain this information.

Later it would be a very simple matter to select one of the cultures which proved most constant in its characteristics and distribute this to all the testing laboratories of a country. In this way the relative toxicity and neutralizing value of the culture fluid grown under the most varying conditions would soon become known.

When we examine the results of the above tests they do not appear to us to reveal the presence of a substance of such a nature as that of the epitoxoids, as defined by Ehrlich, nor do they seem to indicate the possibility of detecting, except in so far as comparative tests suggest, the purity or constituents of a fatal dose of diphtheria culture fluid or "toxin." If epitoxoids have the properties described by Ehrlich, then they should in proportion to their presence increase the gap between L_+ and L_0, but we find no evidence of this in the tables for the fluids having by Ehrlich's formula and theory far the greatest amount of epitoxoids; the deteriorated seven and eight-weeks' toxins have almost the smallest gap between L_+ and L_0. Again we find decidedly more epitoxoids at the end of 22 and 48 hours' growth of the culture 8 than at 6 days and 3 weeks. This certainly seems unlikely, for with the increased amount of deteriorated toxin present in the culture of longer growth in the incubator, we should expect an increase, not a decrease of epitoxoids, if such exist. Again the remarkable variations in number of fatal doses required to neutralize one unit between the 6, 22, 28 and 48-hour toxin are difficult to understand on Ehrlich's theory.

There is indeed, with the use of increased quantities of antitoxin, a larger and larger gap between the amount of toxin which just suffices to produce a slight temporary induration or effect upon the health of the test guinea-pig and the amount which kills upon the fourth day. This difference is about three fatal doses when one-tenth of a unit is neutralized and about eight to fourteen when one unit is employed. In the above tables it will be seen that there is an average of about 8 fatal doses between the amount of toxin which kills a guinea-pig on the fourth day and the amount which kills at a later period from paralysis or increased weakness. This amount is not greater, however, in the old deteriorated toxin than in the fresh, and shows great variations.

This inhibition, through antitoxin, of the otherwise quickly fatal effect of such an excess as six to ten fatal doses of toxin, suggests a difficulty in accepting as proven the chemical theory of the neutralizing effect of antitoxin as being due to its direct combination with the toxin.

Ehrlich not only forms a theory to explain the varying neutralizing value of a fatal dose of toxin, but through a formula, as already stated in the earlier part of this article, he attempts, and, as he believes, attempts successfully, to separate a fatal dose of "toxin" into its constituents and thus detect with accuracy what amount of antitoxin it should neutralize. He claims that by such separation only can a toxin and therefore an antitoxin be properly standardized. Neither his theoretical reasoning nor the results of the working of his formula seem to us convincing of the accuracy of these claims. Not only the weight of Ehrlich's authority, but the practical importance to all those who test antitoxin of his conclusions, demand that we discuss his views.

Upon the four-day limit of life for a guinea-pig which has received a fatal dose of toxin he obtains the values of L_+, L_0, and therefrom the epitoxoids. A dose of toxin which kills most 250-grm. guinea-pigs in four days is for practical purposes called the minimal surely fatal dose for all pigs of that weight. It is, however, an arbitrary standard, and is in fact much more than the minimal fatal dose for most guinea-pigs. A glance at the control pigs in any table—Table IV is a good example—will show how much beyond the actual fatal dose for guinea-pigs is the amount which kills on the fourth day. A minimal fatal dose for a single pig will kill at the end of a week or ten days, or later still by paralysis in the 2nd or 3rd week. This seems so apparent as hardly to need stating.

If, however, Ehrlich should choose such a real limit, or even put it at the sixth or seventh day, it would entirely alter the values of L_+ and L_0, and therefore the results of his formula. The value of L_+ can be fairly accurately determined, but not that of L_0, since slight induration or loss of weight may or may not occur in different animals from the same amounts. His assumption that one unit of antitoxin neutralizes 200 fatal doses of pure toxin or of its derivatives seems to us purely theoretical. Even if the toxin molecules be divided as he believes, still in any given bouillon there is actual destruction, as well as production, going on all the time of all substances, not only those toxic to guinea-pigs, but also of those which, though not toxic, still neutralize antitoxin.

If Ehrlich's formula really revealed the nature of a "toxin," it should answer when employed with larger or smaller amounts of antitoxin than one unit. The L_+, L_0, and the minimal fatal dose should have the same relative value when .1, 1 or 10 units of antitoxin are mixed with varying amounts of toxin. The 200 would merely become 20 or 2000. If, however, we test the formula by solving the same toxin when different known quantities of antitoxin are employed, the amount of epitoxoids will vary with every test; thus toxin from culture Greenwood of three days' growth tested against 1 unit shows:

TABLE XII.

GREENWOOD TOXIN OF THREE DAYS' GROWTH, THE FLUID BEING THE SAME AS TESTED IN TABLE VI AND TESTED AT THE SAME TIME.

	Weight of guinea-pig.	Amount of toxic bouillon in cc..	Amount of serum in cc.	Result in animal.
1.	245	.80	.00012 = .1 unit	Unaffected.
2.	247	.84	.00012 = .1 unit	Died 8th day.
3.	222	.86	.00012 = .1 unit	Died 8th day.
4.	247	.88	.00012 = .1 unit	Died 4th day.

Minimal fatal dose .058 (see Table VI).

$L_{+} = .88 = 15.2$ fatal doses.

$L_{0} = .80 + = 13.9$ fatal doses.

$L_{+} - L_{0} = .08 + = 1.3$ fatal doses.

Epitoxoids in .1 unit $= \frac{20 \times (L_{+} - L_{0} - 1)}{13.9 + (L_{+} - L_{0} - 1)} = \frac{20 \times .3}{13.9 \text{ plus } .3} = .42$

Epitoxoids in 1 unit = 4.2.

Epitoxoids in the same toxin tested by formula when 1 unit of antitoxin is employed = 29.

This table illustrates also that the new method of grading the value on the L_{+} amount with one unit is somewhat more severe than the old method of grading upon the L_0 with one-tenth of an antitoxin unit.

SUMMARY AND CONCLUSIONS.

Until recently diphtheria " toxin " was supposed to be a single definite substance and to have a definite toxicity in animals and neutralizing power for antitoxin. A fatal dose of toxin, without regard to the conditions under which it was produced or preserved, was supposed to require always the same quantity of antitoxin to neutralize it. Ehrlich's researches have completely done away with this theory, and have substituted for it one which assumes the toxin to be only at its origin a single definite chemical compound with definite physiological and antitoxic properties. According to Ehrlich the toxin is an unstable substance which readily loses its toxicity, while at the same time its affinity for antitoxin may be either increased or decreased. Its neutralization by antitoxin he considers to be due to a chemical union between the toxin and the antitoxin.

The results of our experiments as detailed in this paper are fully in accord with those published by Ehrlich, as to the varying neutralizing value of a minimal fatal dose of "toxin"; they, however, go further and indicate roughly a general law in accordance with which these changes occur.

The neutralizing value of a fatal dose of toxin is at its lowest in the culture fluid when the first considerable amounts of toxin have been produced. After a short period, during which the quantity of toxin in the fluid is increasing, the neutralizing value of the fatal dose begins to increase, at first rapidly, then more slowly.

While the culture is still in vigorous growth and new toxin is being produced, the neutralizing value of the fatal dose fluctuates somewhat, but with a generally upward tendency. After the cessation of toxin production the neutralizing value of the fatal dose increases steadily until it becomes five to ten times its original amount.

In our experiments the greatest value for L_+ was 126, the least 27. As at 6 hours L_+ was only 72 and at 28 hours only 91, we doubt whether L_+ ever reaches above 150, and therefore hardly expect Ehrlich's figures of 200 to be realized. When we seek to analyze the above-described process, we find certain facts which seem partly to explain it. Experiments have shown that filtered toxin, preserved for any length of time in conditions under which access of air occurs, gradually loses in both its toxicity and neutralizing power, and that it loses more rapidly in the former property than in the later. Thus, while the fatal dose of a toxin preserved for one year rose from .01 cc. to .55 cc., it lost only half as much in neutralizing value, one unit neutralizing at first 1 cc., at the end of the year 25 cc. These processes take place more rapidly at room temperature than in the ice chest, and in the incubator than in the room.

In the fluid holding the living bacilli we have, therefore, after the first few hours of toxin formation, a double process going on, one of deterioration in the toxin already accumulated, which tends to increase the neutralizing value of the fatal dose, the other of new toxin formation, which probably tends to diminish the neutralizing value. The chemical changes produced by the growth of the bacilli in the bouillon tend to aid one or the other of these processes and so to make from hour to hour slight changes in the value of the fatal dose. Later, with the period of cessation of toxin production, the gradual deterioration of the toxicity alone continues, and the fatal dose gradually and steadily increases in its neutralizing value.

Ehrlich's theories, as to the splitting up of "toxin" into toxoids having little or no toxicity but on the average full neutralizing power for antitoxin, have not in our opinion been substantiated by the results of these experiments. The difference between the amount of toxin mixed with a unit of antitoxin which causes the first symptoms and that causing death upon the fourth day would be, it is true, explained by his theory, but the failure of this difference to be greater where, by his theories, epitoxoids should be in great abundance prevents our acceptance of his views. The fact of the greater neutralization value of a fatal dose of a deteriorated toxin would be accounted for on his protoxoid theory. This, however, is not proof of its correctness, as other theories, such as the production by the diphtheria bacillus of two or more closely allied toxins, similar to the allied alkaloids produced by plants, would equally account for it, if we supposed the one which had the greater neutralization value was more resistant to destruction than the other. We only advance this theory to call attention to the fact that many theories can on paper explain a process without necessarily being thereby established.

Even if his theories prove partially correct, we feel certain that his formula for standardizing toxins is founded upon error and cannot be employed for the purpose intended by him.

While we do not believe, therefore, that he has changed the principles of testing antitoxin, yet we believe he has contributed greatly to uniformity in results by calling attention to the necessity of selecting a suitable toxin and by employing and distributing an antitoxin as a standard to test toxins by. In this way smaller testing stations can make their results correspond with those of the central station.

In spite of the great variations in the neutralizing value of a fatal dose in different toxins, we do not believe there has been any such great difference in the toxins used by the different stations for testing purposes. Most laboratories have taken the culture fluid at about the time of its greatest toxicity, and the neutralizing value of a fatal dose of this toxin would seldom vary more than 10 per cent above or below the standard now adopted in Germany by the government testing station, this latter being presumably as close as possible to that used to establish the original Behring-Ehrlich unit.

Where error has been made, it has usually been by taking too old culture fluids, which would cause the antitoxin strength of samples tested to be estimated below and not above its real value. Culture 8, which is used not only by us but by many other laboratories in the United States and Europe, fortunately produces on the 6th day, the time at which the culture is usually removed, a toxin which grades Ehrlich's antitoxin within five per cent of the strength given by him.

We believe that by using such a bacillus, we can, after gaining a fuller knowledge of its characteristics, obtain a toxin of a known and suitable neutralizing value, and thus always correctly standardize an antitoxic serum. Meanwhile a fairly permanent antitoxin, such as Ehrlich provides, is of immense value in insuring a uniform though not necessarily correct standard among the different testing stations and in allowing of comparison between them.

AN EXPERIMENTAL STUDY OF THE DIRECT INOCULATION OF BACTERIA INTO THE SPLEEN OF LIVING ANIMALS; AND A CONTRIBUTION TO THE KNOWLEDGE OF THE IMPORTANCE OF A LESION IN ANIMAL TISSUE FOR THE LODGMENT AND MULTIPLICATION OF BACTERIA WITHIN IT.

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The methods of experimental infection which are at the present time mostly used are subcutaneous or intraperitoneal inoculation or intravenous injection of the micro-organisms employed. The final object to be attained is the introduction of the organisms into the circulation, and the different results obtained by these various methods of inoculation are largely due to a mechanical factor—to the rapidity with which the organisms and their products are absorbed from the locality in which the injection was made.

It occurred to the writers that new characteristics in the forms of infection might be obtained from a systematic study of inoculations made primarily into one of the internal organs.

It seems fair to suppose that specific metabolic processes take place in all organs having special functions, and it is not illogical to assume that the specific catabolic processes of an organ might have a characteristic effect upon bacteria, more particularly when they are unchanged by previous contact with the blood or lymph.

The spleen, above all the internal organs, seems to harbor the specific micro-organisms in certain of the infectious diseases, and for this reason, notwithstanding the fact that the exact function of the spleen seems to be little understood, this organ was selected for our experiments as the seat of direct infection. From its peculiar anatomical structure, some hold the opinion that the spleen acts as a filter and holds back bacteria from the blood passing through it. It might also be claimed that the bactericidal power of the blood is increased in the spleen, for on entering the organ, the blood leaving the capillaries comes in close contact with the lymphoid tissue where leucocytes abound, and it is quite generally believed that a close connection exists between leucocytes and the bactericidal action of body fluids.

Now, on the other hand, H. Buchner* claims that wherever disintegration of the red blood corpuscles occurs, the alexines fail to display their full power; and according to the prevailing opinion the erythrocytes are broken down in large numbers in the spleen. Hence it might be assumed, as a fair hypothesis, that while the non-corpuscular alexines are easily carried from the spleen by the blood, that the corpuscular elements, including the micro-organisms, remain in the spongy tissue of the spleen, and the bacteria, well nourished by the broken down erythrocytes, resist the attacks of the remaining alexines.

Our first problem then was to determine the fate of bacteria when inoculated directly into the spleen.

The animals experimented on were chiefly rabbits; the inoculations were mostly of broth cultures of B. coli communis, and the results obtained were controlled by inoculations of broth cultures of B. typhosus and Staph. pyogenes aureus. The inoculations, made with a hypodermic syringe, were directly into the substance of the spleen, the needle being introduced into the ventral end, *i. e.* that end of the spleen lying nearest the abdominal wall. The operation for exposing the spleen was made under ether anesthesia, the incision in the median line of the abdomen causing little or no loss of blood. In none of our numerous experiments did a secondary infection at the site of operation occur, the wound in the abdominal wall healing *per primam.*

The slight bleeding from the spleen, caused by the puncture of the hypodermic needle, was arrested either by a ligature thrown around the needle or by the actual cautery.

In due time each animal was killed with chloroform and the autopsy performed immediately under strict aseptic precautions. After opening the thorax and abdomen the spleen was at once removed and placed in a sterilized Petri dish, and cultures were then made in 10 per cent nutrient gelatin, reacting 1.5 per cent acid to phenolphthalein, from the blood in the left ventricle, hepatic vein, liver and left kidney, as a routine procedure; other cultures being made from time to time as hereafter noted. Gelatin plates were then made from the spleen, which for convenience was divided into three parts; the ventral end, or that through which the inoculation was made, being designated I, the middle third, II, and the dorsal third, III. Three large "loops" of the juice of the spleen were used for making the plates and other plates were made with bits of tissue torn out from the substance of the organ. All plates were kept under observation for not less than seven days. Cover-glass smears were also made from the tissues plated.

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* *Centralbl. f. Bakt. u. Parasit.*, vi, No. 1.

*Experiment 1.** Medium-sized rabbit; 5 minims of 24 hours broth culture of B. coli communis injected into spleen. Autopsy after 40 hours. Plates from parts I, II and III of the spleen remained sterile.

Experiment 2. Small rabbit; 6 min. of 48 hours broth culture of B. coli comm. injected into spleen. Autopsy after 40 hours. Spleen plates; I showed 4 colonies; II, 2 colonies; III, 5 colonies. Plates from liver, 6 colonies; from hepatic vein, 2 colonies; and from left ventricle, 4 colonies.†

Experiment 3. Small rabbit; 6 min. of 24 hours broth culture of B. coli comm. injected into spleen. Autopsy after 14 hours. Spleen plates; I, 3 colonies; II, 7 colonies; III, 0. Plates from liver, 0; from kidney, 1 colony.

Experiment 4. Large rabbit; 10 min. of 24 hours broth culture of B. coli comm. injected into spleen. Autopsy after 14 hours. Spleen plates; I, 11 colonies; II, 43 colonies; III, 3 colonies. Plates from liver, 32 colonies; from hepatic vein, 15 colonies; from left ventricle, 133 colonies; and from left kidney, 0.

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* The experiments are numbered in the order in which they are reported, not in the order in which they were made.

† The number of colonies reported are the average of two or more plates made from the same locality, the number occurring on each of the several plates not being far from the average obtained.

Experiment 5. Small rabbit; 5 min. of 20 hours broth culture of B. coli comm. injected into spleen. Autopsy after 3 hours and 20 minutes. Spleen plates; I, 0 colonies; II, 13 colonies; III, 3 colonies. Plates from liver, 2 colonies; from hepatic vein, 0; from left ventricle, 5 colonies; and from left kidney, 0.

Experiment 6. Small rabbit; 25 min. of 4 days broth culture of B. coli comm. injected into spleen. Rabbit died in 3½ days; autopsy six hours after death. Spleen plates; I, 200; II, 350; III, 150 colonies of B. coli communis; no other colonies present.

Experiment 7. Large rabbit; 25 min. of 48 hours broth culture of B. coli comm. injected into spleen. Died after 24 days. Plates from the three parts of the spleen, from the liver, hepatic vein, left ventricle and left kidney all remained sterile.

It seems unnecessary to mention further experiments of this kind; the few here quoted from our protocols show that large numbers of bacteria injected into the spleen soon disappear.

The organisms injected into the spleen were neither kept back mechanically, as in a filter, nor did they find within the spleen a soil favorable to their multiplication. Although the 5-minim dose of broth culture, which was injected, corresponds to about one-third of the entire volume of the spleen and contained many millions of bacteria, the plates made from the juices and tissue of the spleen were for the most part sterile, and even in the plates made only 3 hours and 20 minutes after the inoculation, but few colonies developed. Controls of the cultures employed for the inoculations were made in several instances by adding a small loopful of the culture to 5 cc. of sterile broth and immediately plating three loopfuls of this mixture in nutrient gelatin. The number of colonies developing on these plates varied from 3000 to 10,000.

These control experiments assured us that many millions of bacteria disappeared from the spleen within a short time.

The question now arose: Is this large number of bacteria rapidly destroyed in the spleen, or are these bacteria carried out from the spleen in immense numbers by the blood current?

The best method to exclude the latter proposition would have been to tie all the splenic veins, but this we could not do in the small animals experimented with. In a single case an attempt was made to prevent the flow of blood from the spleen by tying the portal vein.

Experiment 8. Medium-sized rabbit; ligated portal vein and then injected 5 min. of broth culture of B. coli comm. into the spleen. Animal died in 30 minutes. The spleen was removed, placed in a sterilized Petri dish and plated 14 hours later. Juices and tissue were plated from five different incisions made into the spleen. All plates showed an innumerable growth of B. coli and no contaminations were found.

In the other experiments it seemed preferable to tie the splenic vessels themselves, but we were obliged to ligate the arteries together with the veins.

Experiment 9. Large rabbit; tied all splenic vessels and injected into the spleen 5 minims of broth culture of B. coli communis. Autopsy after 40 hours. Spleen plates I, II, III all show an innumerable growth of B. coli communis.

Here the bacilli could not migrate into the circulation, and indeed there was every evidence of an active growth of them within the spleen. We have to remember, however, that the supply of fresh blood and with it the supply of fresh alexines were cut off, while the amount of alexines present in the spleen at the moment of ligating the vessels was probably out of proportion to the number of bacteria introduced into the spleen. It is now generally accepted that the effect of the bactericidal elements in the blood is but little apparent in the presence of large numbers of bacteria, and that after the bactericidal powers are exhausted the surviving bacteria multiply with great rapidity.

Anatomists teach that the capillaries in the spleen resolve and the blood enters the tissue; and the assumption seems justified, and is indeed tacitly made, that quite a fair communication exists between the interstitial spaces of the spleen. We therefore resorted to the plan of ligating only a part of the vessels pertaining to the spleen, anticipating that the migration of bacteria from the ligatured portion would be considerably restricted, while there might be some, perhaps an abundant supply, of the bactericidal factors to this part provided by the neighboring parts of the spleen through which the blood was allowed to flow.

Experiment 10. Small rabbit; tied about ⅓ of the vessels on the ventral end of spleen, and through the ventral (ligated) end injected 5 min. of a 24 hours broth culture of B. coli comm. into the middle portion of spleen. Tip of spleen tied. Autopsy after 40 hours. Tip of spleen was found detached from the rest of the organ and imbedded in the mesentery; the ventral third of the spleen, the vessels of which had been ligated, was considerably thickened and paler than the remaining part of the organ, and distinctly separated from it by a ridge or line of demarcation. The plates made from the ligatured part of the spleen (I) were overcrowded with colonies and the plates from parts II and III showed growth of colonies which were too abundant to count. Colonies were most abundant in plates from part I, distinctly less abundant in plates from part II, and still less numerous in the plates from part III.

Experiment 11. Medium-sized rabbit; blood-vessels of the ventral third of spleen ligatured and 6 min. of a broth culture of B. typhosus injected through the ventral end into the middle third of spleen. Autopsy after 40 hours. The ligated end of the spleen was hard, dark red and dull on the surfaces, and the hard portion was separated from the normal part by a sharp ridge. Spleen plates; I, innumerable colonies; II, 1900 colonies; III, 1150 colonies. Plates from liver developed 91 colonies; from hepatic vein, 90 colonies; from left ventricle, 20 colonies; and from left kidney, 4 colonies, of B. typhosus.

In some experiments, in which the vessels of the middle portion of the spleen were ligated and the animal was allowed to live for seven or eight days, the entire middle portion of the spleen had become very much thickened, of the greyish yellow color and remarkably dry, while both ends of the organ, the circulation in which we endeavored not to interfere with, appeared normal in every respect.

Thus we see that our expectation of a collateral interstitial nutrition was not realized, an interesting fact which, however, we cannot discuss further here.

On the other hand, our experiments show that plates from the non-ligated portions of these partly ligatured spleens developed a considerable number of colon or typhoid colonies, quite in contrast to our experience with spleens no part of which were ligated.

In studying over our entire material it seemed to us that the assumptions which give the fittest interpretation of all the facts in our possession—at least for the present—are: (1) That the blood within the spleen is less bactericidal than the blood within the balance of the circulation, on account, perhaps, of the nourishment afforded the bacteria by the large numbers of disintegrated erythrocytes within the spleen; and (2) That the structure of the spleen offers but little hindrance to the rapid migration into the circulation of large numbers of bacteria injected into this organ.

Our experiments, which showed that but few bacteria could be found in the spleen only a few hours after large numbers had been injected into it, must be explained by assuming that great numbers of them were soon carried away from this organ through the splenic veins by the blood stream; the fate they met was probably that which awaits those large numbers of bacteria which are injected directly into the circulation.

In our second set of experiments, in which the bacteria were injected into a spleen, part of the vessels of which had been ligated, the presence of bacteria in the non-ligated (normal?) portion of the spleen may be explained by assuming that many of those which got into the ligated part remained there and, from the abundance of nutriment, multiplied rapidly. From this source a fresh supply of bacteria was constantly given out to the adjacent, more normal part of the spleen, whence the blood, flowing through the organ, carries the bacteria out almost as soon as they are supplied to it.

Although it seems to be a fact that but little interchange of nutritive fluids takes place between the several regions of the spleen, the bacteria are apparently able to wander over by their own motility (colon and typhoid), or to grow through, from one region into another.

The fact that, only a few hours after injection into the spleen, so few bacteria could be found in the blood, does not argue against the assumption that all the injected bacteria were carried out into the blood stream, because they disappear in the same manner when injected directly into the veins.

Wyssokowitsch * found five hours after injecting 1 cc. of typhoid

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* *Ztschr. f. Hygiene*, i (1886), 1.

culture into the jugular vein of a rabbit that 0.2 cc. of the blood contained only 5 bacilli.

The presence of 1900 colonies in plates made from a point in the spleen adjacent to a part the vessels of which had been tied, while not more than 90 colonies were found in plates made from the liver and the hepatic vein, may well be interpreted in favor of the assumption that blood within the spleen is less bactericidal than blood in the vessels nearest to this organ.

Another point in favor of this assumption seems to be the fact that the bacteria thrive in a ligated spleen, while, according to de Giaxa and Guarnieri,* bacteria succumb to the bactericidal effects of blood in the vessels between two ligatures as well as in the extra-vascular blood.

Finally, we have to report that in a number of cover-glass smears made from non-ligated spleens, which were most carefully examined microscopically, no bacilli were discovered; and it would seem to us improbable, even if only a considerable portion of the bacteria were killed by the alexines, that no bodies of the bacteria, which would take a stain, should be found in the smears.

The views here put forward would seem not to be in harmony with the statements of Wyssokowitsch.† This author found, after injecting numerous micro-organisms into the jugular vein, that they soon disappeared from the circulation, but that a number of them settled in the spleen, liver and bone-marrow, a fate which, according to Ponfick ‡ and others, they share with non-organized corpuscular bodies, such as cinnabar, ultramarine, etc., injected into the circulation.

Wyssokowitsch, however, states further that certain bacteria, for example the typhoid bacillus and others, soon become destroyed within the spleen. He states that twenty-four hours after injection of the typhoid bacillus into the blood, cultures made from the spleen show no living bacilli; in other words, he claims that the spleen acts as a filter upon the organisms in the blood passing through it, and the bacilli succumb to the bactericidal influences within the spleen.

With regard to the first point we freely admit that corpuscular elements may find within the spleen a greater obstacle to their free movement than in any other part of the blood current, and that a larger accumulation of organisms may occur in the spleen than in any other part of the circulation; yet the results of our experiments have prompted us to assume that the retained bacteria are easily carried out again from the spleen, and that the " filtration " is comparatively of but little account and not permanent. As to the disappearance of the bacteria from the spleen, we maintain that Wyssokowitsch has not offered the slightest proof of his assumption that the destruction of the bacteria occurs within the spleen. This disappearance of the bacteria can be as well explained by supposing that they have been gradually carried out from the spleen by the blood into the vessels, where they have succumbed to the bactericidal power of the circulating blood. We wish it understood that we by no means claim that the spleen possesses no degree of bactericidal influence and that no destruction of bacteria takes place in this organ. On the contrary, at the beginning of our experiments, the total sterility of the inoculated spleens led us to believe that the bacteria were destroyed by the bactericidal elements of this organ, and when during our investigations the highly important publications of R. Pfeiffer * and of A. Wassermann † appeared, showing that the elements of active immunization in cholera and in typhoid fever appear in the greatest amounts in the spleen, lymphatic glands and bone-marrow; and inasmuch as immunization in typhoid fever and in cholera does not mean antitoxic but bactericidal effects, we were further strengthened in our belief that we, too, had found bactericidal effects of remarkable power within the spleen. Yet a careful analysis of the results of all our experiments led us to the present conclusion that the bactericidal power within the spleen acts rather less intensely than in the rest of the circulation.

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* *Annales de Micrographie*, iii (1890-91), 481.

† Loc. cit.

‡ Virchow's *Archiv*, 1869.

As a matter of fact, this view seems to us to harmonize far better with the principles and findings of Ehrlich, Pfeiffer and Wassermann. The normal bactericidal elements are not identical with the bacteri-

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* Pfeiffer and Marx, *Deutsch. med. Wochenschr.*, 1898, p. 47.

† *Berliner klin. Wochenschr.*, 1898, p. 209.

cidal bodies of active immunization; they are derived from different sources. The bactericidal elements of the normal blood are seemingly generated in one way or another by the leucocytes, while Pfeiffer has established the fact that the bactericidal bodies generated by immunization stand in no relation to the leucocytes. Following the theory of the so-called "side-chain-immunity" of Ehrlich,* it is assumed by Behring † and by Wassermann that these bactericidal bodies are generated by the organ-cells, which, reacting to certain stimuli, produce an excess of these bodies. The stimulating agents are the bacteria and their products.

This, in a few words, is the essence of the views of these writers so far as concerns us here. We believe now that we are not going too far when we add the hypothesis that the greatest overproduction of the bactericidal bodies occurs in these organs, in which the bacteria are permitted to multiply most actively. In other words, when the bactericidal elements of the normal blood are less active, less influential, then the most active generation of bactericidal bodies of immunization takes place. It is in this sense, then, we can claim that our assumption of the diminished effect of the normal bactericidal action within the spleen harmonizes with the statements of Pfeiffer and of Wassermann, that the bactericidal bodies of immunization are abundantly produced within the spleen.

In one of our experiments with injections into the non-ligated spleen, numerous bacilli were found to be present in the liver.

In this case an attempt had been made to reach the vagus nerve below the diaphragm, requiring some pressure upon the liver, and it occurred to us that the mechanical injury the liver had received might have been the cause of the retention and perhaps multiplication of the bacteria within it. We therefore attempted to produce by cauterization circumscribed lesions of the surface of the liver before injecting bacteria into the spleen, and our experiments in this line show conclusively that by mechanical injury to the tissue we have it in our power to cause bacteria which are circulating in the blood to settle in any part of the surface of the liver we may select. In all the cases experimented upon the cauterized part of the liver contained innumerable organisms; in the normal part of the organ immediately adjoining the lesion they were still numerous, while in remote parts but few, if any, were to be found. The organisms found in the lesions were pure cultures of the species which had been injected into the spleen. In cases in which the animal was allowed to live for 30 to 40 hours, it was easy to establish the fact that an innumerable quantity of bacteria were present in the lesion, while none were found at the site of inoculation. The perfect sterility of the spleen and the immense number of bacteria found at the site of the lesion showed that at least some of the bacteria migrated from the spleen in a living condition.

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* Die Wertbemessung des Diphtherieheilserums, *Klinisches Jahrbuch*, vi (1897).

† *Deutsch. med. Wochenschr.*, 1898, p. 68.

The liver, however, is the next station at which bacteria escaping from the spleen would arrive, and the question suggested itself whether a sufficient number of bacteria escaped from the spleen to allow of their reaching and settling in a more remote organ, and whether or not lesions of other organs would react in a similar manner to those of the liver.

To this end lesions were produced by cauterization in several organs: in the kidney, the uterus, the testicle, and in a circumscribed area in the peritoneum, and in the subcutaneous tissue, etc. In all places where suitable lesions were produced, the specific organisms were present in large numbers, as illustrated by the following experiments.

Experiment 12. Large rabbit; cauterized pregnant uterus through incision in abdomen and injected 2.5 min. of a 3 days broth culture of B. coli comm. into spleen. Autopsy after 3½ days. Spleen plates; I, 1 colony; II and III, 0. Plates from liver, 0; from hepatic vein, 1 colony; from left ventricle, 1 colony; from left kidney, 0; from the site of lesion in the uterus and from the fluid in the cavity of the uterus at site of lesion, innumerable colonies.

In this experiment the quantity of culture injected was small, the rabbit and the spleen were both large, and the plates made from the blood and uninjured organs were sterile—conditions which would favor the interpretation that the bacteria were all destroyed in the spleen, but the abundant growth in the lesion of the uterus demonstrates that a considerable number of bacilli must have escaped into the general circulation to found this thriving colony in a remote region.

Experiment 13. Medium-sized rabbit; cauterized right kidney and uterus, and injected 5 min. of a three days broth culture of Staph. pyogenes aureus into the spleen. The tip of the organ through which the needle was inserted was tied. Autopsy after 40 hours. Spleen plates from tied tip showed 460 colonies; from part I, 192 colonies; part II, 7 colonies; part III, 4 colonies; plates from the liver, hepatic vein, left ventricle and left kidney were sterile; from the right kidney, necrotic portion and adjoining portion, innumerable colonies; and from the necrotic portion of the uterus over 10,000 colonies developed. The tip of the spleen contained a considerable number of bacilli, as did also the adjoining part, a fact which was observed in nearly all experiments in which the tip of the organ was tied, and which probably is explained by the cutting off of the blood supply by the ligature.

Our observations on the action of bacteria in lesions made in the internal organs seemed to be of such importance, that we conducted a series of experiments by injecting bacteria into the general circulation, after producing lesions in one or more of the internal organs.

Experiment 14. Large rabbit; ventral end of spleen and border of liver squeezed with hot forceps; 25 min. of a 20-hour broth culture of B. coli comm. injected into ear vein. Autopsy after 40 hours. The cauterized portions of the spleen and of the liver were necrotic. Plates from the blood in left ventricle showed 1 colony; from left kidney, 0; from hepatic vein, 48 colonies; from normal part of liver, 2 colonies; from the necrotic part of the liver the colonies were innumerable; from part I, of spleen, the necrotic portion, innumerable colonies; from part II the colonies were also innumerable, but distinctly fewer than were found in part I, and from part III only 39 colonies developed.

Experiment 15. Large rabbit; cauterized right kidney, border of liver, two areas in spleen, one near each end, a circumscribed area of the peritoneum, and the subcutaneous tissue in the right inguinal region. Twenty-five minims of a broth culture of B. coli communis was then introduced into the ear vein and the animal killed after 64 hours.

Plates made from blood in the left ventricle showed no growth; from hepatic vein, 0; from a normal portion of the liver, 0; from necrotic part of the liver, innumerable colonies; spleen plates showed an innumerable number of colonies from the necrotic portions and a very abundant growth, though distinctly less, from the intermediate parts; plates from the left kidney showed no growth, while those from the normal part of the right kidney showed 410 colonies; the development from the necrotic portion of the right kidney and the apparently healthy tissue adjoining it was innumerable, though distinctly less in the latter case than in the former. Plates made from the cauterized portion of the peritoneum and from the necrotic subcutaneous tissue, all showed an innumerable growth.

Experiment 16. Medium-sized rabbit; squeezed with hot forceps the ventral end of the spleen, the lower border of the liver and the lower end of the right kidney. Twelve minims of broth culture of B. coli comm. were then injected into the ear vein. The autopsy was made after 41 hours and plates from blood in the left ventricle developed no colonies; from hepatic vein, 72 colonies; from normal part of liver, 570 colonies; from the necrotic part of this organ, innumerable colonies; from an adjoining portion, 2671 colonies; from the left or normal kidney, 0; from the squeezed portion of the right kidney, innumerable colonies. Plates made from various parts of the spleen developed very few colonies.

The few colonies found in plates from the spleen may perhaps be explained by the fact that the squeezed portion of the spleen was dry and leathery in appearance and very tough when cut into, and it may be that the circulation was so completely cut off from this part that the bacteria had but little chance to reach the lesion in the organ, and were mostly carried out again into the circulation. It seems probable that only when some more or less normal tissue remains within the necrotic part, *i. e.* tissue through which the blood can flow, that the bacteria can gain access to the injured tissue and be deposited there. It is of course a very difficult matter always to regulate the amount of pressure exerted, so as to produce the same kind of a lesion in every experiment.

In this connection it seems important to mention that in experiments in which half of the vessels of the spleen were tied and the inoculation made into the circulation through the ear vein, plates made from the spleen, whether from the ligated or the non-ligated part, remained sterile. The sterility of the ligated portion probably finds its explanation in the reasons set forth above. Injections made directly into or through a ligated portion of the spleen offer entirely different conditions, for here the bacteria are brought directly into the ischæmic part and could not escape except through their own motility or by growing out through enormous multiplication. It seems likely that the abundant growth within the ligatured portion of the spleen was due largely to the lesion of the tissue caused by ligation of the vessels which favored the retention and development of the organisms.

The principle underlying the fact that a lesion in an organ favors the retention and development of bacteria within it is not new. J. Rosenbach,* Becker,† and others, have shown that the intravenous injection of Staph. pyogenes aureus can cause osteomyelitis in a fractured bone. The experiments of Wyssokowitsch,‡ and of Prudden,§ have established that a mechanical lesion, and according to Prudden that also a chemical lesion, of the cardiac valves causes the staphylococcus and the streptococcus, when intravenously injected, to settle there and develop ulcerative endocarditis. According to Wyssokowitsch, however, the M. tetragenus and other organisms did not settle in the injured valve. In our experiments with the three species of bacteria mentioned, we made no attempt to determine how they would act in relation to injured heart valves, but were enabled to cause them to settle in any tissue experimented with, by producing a suitable lesion. This seems to us a point of great practical importance, inasmuch as any physiological or artificial injury, such as parturition or operation, carried out under the most scrupulous asepsis, can become infected, if in any place in the body there exists a focus containing micro-organisms; and in this connection it must not be forgotten that attenuated micro-organisms may regain their virulence in a tissue lesion.|| How many cases of puerperal infection or of post-operative

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* *Zeitschr. f. Chirurgie*, x.

† *Deutsche med. Wochenschr.*, 1884.

‡ Virchow's *Archiv*, ciii.

§ *Amer. Journ. Med. Sciences*, 1887, p. 70.

|| Nocard and Roux, *Annales de l'Institut Pasteur*, 1887.

sepsis might here find their explanation! Without, however, entering into a discussion of the numerous instances in pathology which can be interpreted profitably by our facts, we would add that, judging from a few experiments we have made, it would appear that lesions of the internal organs can become infected also from a subcutaneous focus.

In one experiment in which subcutaneous injection of the B. coli comm. was employed, plates made from the injured portion of the liver contained over 2000 colonies, while the necrotic spleen showed a very meagre growth. In another similar experiment the plates from the necrotic portion of the spleen contained over 6000 colonies, from a cauterized portion of the peritoneum about 1000 colonies, and in plates from the cauterized uterus there were innumerable colonies.

The failure of Wyssokowitsch to produce ulcerative endocarditis from subcutaneous injections of pyogenic bacteria may perhaps be explained by the fact that certain bacteria are but slowly absorbed from the subcutaneous tissue; if the injury to the valves had been caused a few days after the injection instead of simultaneously, more time would have been allowed for the bacteria to get into the circulation, and the result might have been different.

The frequent mention of the presence of B. coli comm. in the cauterized portions of the liver might lead to the suspicion that this ubiquitous organism was present in the liver before the intrasplenic or intravenous injections were made. Aside from the numerous instances in which the uninjured organs were found free from this bacillus, other studies bearing directly on this point were made. A number of rabbits were opened, the liver and spleen were cauterized and the abdomen closed, just as in the other operations alluded to, but without any injection of micro-organisms whatever. After a few days the animals were killed, and plates made immediately from the injured and uninjured organs and from the blood, and in all the cases the plates were found to be sterile. It therefore seems to us probable that the cases in which numerous colon bacilli were found in the normal liver were from platings made some time after death had occurred.

In conclusion we desire to say that, although in our many experiments we have produced numerous foci containing very many bacteria, and thus have offered a number of different sources for the abundant supply of bacteria to the blood, the blood, nevertheless, has remained perfectly sterile or contained but very few bacteria. The blood is a liquid tissue, and may we not, perhaps, assume that the blood, like other tissues, permits the growth of bacteria within it only when it has been in some way injured?

SUMMARY.

I. Cultures of B. coli communis, B. typhosus and Staph. pyogenes aureus, when injected into the tissue of the normal spleen, soon disappear from that organ, and indeed from the normal body generally.

II. Bacteria injected into a spleen after the whole or a part of the vessels have been tied, multiply in the spleen with great rapidity and continue to supply bacteria to the blood, whence in the healthy body they soon disappear.

III. Bacteria injected into the spleen, or subcutaneous tissue, or into the blood current through the ear vein, in cases in which moderate lesions have been made by cauterization or compression in the spleen, liver, kidney, uterus, testicle, peritoneum, or subcutaneous tissue, usually find lodgment in these lesions and multiply there.

IV. Even in cases in which numerous foci existed, from which the blood was constantly provided with a fresh supply of bacteria, only few bacteria were found at any time in the blood.

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ON A FORM OF DEGENERATION OF STRIATED MUSCLE MET WITH IN THE UVULA.

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PLATES XLII–L.

There is but little literature descriptive of the microscopic morbid anatomy of the uvula. This is true especially of the condition known as relaxed or elongated uvula. Inman* in 1852 described the uvula in this condition as being "very firm and almost fibrous in the interior, covered with ordinary mucous membrane, which was not hypertrophied. On making sections and teasing with needles, the structures were found to consist of groups of large cells, 1/250 inch in diameter, studded with an immense number of granules." These he supposed to be altered cells from the mucous glands, bound together by strong areolar tissue. "Throughout this tissue were interspersed numerous muscle fibres, arranged in bundles, and but few of the fibres had the characteristics of voluntary muscle. Very few were striated; all of them seemed to be composed of an outer membrane and an inner substance, closely resembling fibrin. It was homogeneous, yellow, divided irregularly into fragments by transverse divisions. The majority of the fibres terminated in blunted points as if they were undergoing absorption."

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* *Medical Times and Gazette*, 1852, v, p. 232.

The condition of the uvular tissues found by Inman was, in the main, probably the same as that which I shall describe in this article. It is not unlikely that, owing to the imperfect microscopic technique and methods of staining and examination at the time when Inman wrote, he was unable to study the exact relations of the tissues and to interpret correctly what he saw.

I have recently had the opportunity of studying the uvula microscopically in certain pathological conditions, and of comparing the appearances found with those in normal specimens.

The material serving as a basis for this study consisted of the following: (1) a uvula, relaxed and elongated, removed from a patient in my own practice and fixed for microscopic examination in Zenker's fluid; (2) three uvulæ from the Laryngological and Rhinological Clinic of the Johns Hopkins Hospital Dispensary, removed by Dr. Gamble, which were hardened respectively in saturated mercuric bichloride (aqueous) solution, 5 per cent formalin, and Zenker's fluid; of these three specimens the formalin preparation proved to be practically normal and served as a standard for comparative purposes; (3) a fifth uvula, removed by Dr. Mackenzie for elongation, from a case of chronic pharyngitis in an inveterate smoker, and hardened in graded alcohols; (4) the sixth removed by Dr. Flexner at autopsy from a new-born infant half an hour post mortem and fixed in 5 per cent formalin. Obligations are here expressed to the above-named gentlemen for their courtesy in securing and sending the material as enumerated.

In May, 1896, R. W., æt. 30, presented himself for treatment, fully impressed with the idea that he was suffering with a tubercular affection of the lungs. Mother died of phthisis at 40. Family history otherwise good. Patient is a moderate smoker. Briefly stated, his symptoms were as follows: He suffered from cough which was of an irritating character and incessant, and he had very scanty expectoration of a rather tenacious and glairy mucus. Occasionally this became puriform. No hæmoptysis. He had morning vomiting and he was also sick at his stomach during the day. His temperature in the evening was slightly elevated (99°-99.5° F). The appetite was good, but the irritating cough frequently caused regurgitation or vomiting of his food. His sleep was very much broken by the cough, and he was compelled to use an anodyne cough-mixture. Deglutition was painful, and there was a constant desire to swallow.

Inspection of his throat revealed a moderate erythematous blush involving the whole of the pharyngeal mucous membrane, as well as the pillars and arch of the soft palate. The mucous membrane seemed to be slightly œdematous and presented a moderate degree of capillary injection. The uvula was extremely long and relaxed, and rested, curled up, on the base of the tongue. Examination of the sputum was negative as to tuberculosis; auscultation and percussion demonstrated little of note, a slight harshness on forced inspiration and expiration being the only abnormality perceptible. Circulation sound and renal functions perfect.

As most of the symptoms seemed to point to a reflex origin due to the elongated uvula, abscission of the latter was advised and performed, with prompt relief of the urgent symptoms.

Microscopic Technique.—The removed portion, somewhat over 2 cm. in length, was immediately placed in Zenker's fluid, where it remained for 36 hours. It was then transferred to 80 per cent alcohol, and finally run through absolute alcohol, thin and thick celloidin, in which it was imbedded. Longitudinal sections were made. An oversight occurred in neglecting to treat the sections first stained and mounted with iodine solution, in consequence of which these show precipitates of the mercurial salts.

Various staining methods were employed, Delafield's hæmatoxylin solution, and one* which I have employed for some time in this laboratory, giving on the whole the most satisfactory specimens for general purposes. The sections stained in Delafield's hæmatoxylin were in some instances differentiated by a short immersion (from 1 to 2 minutes) in an aqueous solution of oxalic acid (1 per cent); in others, in a saturated solution of picric acid in water for the same length of time. Sections stained in the glycerine-Heidenhain-hæmatoxylin mixture require no differentiation, as it does not overstain, and is quite elective in its action, requiring, however, from 12 to 24 hours before staining is complete. For counterstaining a saturated alcoholic (95%) solution of ammon. picrate, to a watchglassful of which a few drops of a 2 per cent aqueous mixture of liq. ammoniae were added, was used, the sections being transferred from the acid differentiating bath directly to this. Eosin-creosote (from 2 to 4 per cent sol.) was also used as a counterstain and as the final clearing agent for some of the sections, but the ammonium picrate treatment gave by far the most satisfactory and instructive specimens.

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* Glycerine, 50 cc.; Heidenhain's hæmatoxylin sol., 20 cc.; alcohol, 95 per cent, 100 cc.; water, 50 cc.; ammon. alum, 2-4 grammes.

The sections were transferred directly from the ammon.-picrate-alcohol solution to the slide, where they were dried with filter paper, and immediately flushed with xylol-anilin for a second or two, then with pure xylol, and finally mounted in balsam. Terebinthinated creosote, cedar and clove oils were also employed as clearing agents, and after special staining methods. Weigert's fibrin stain, van Gieson's picric acid-fuchsin, Ehrlich's triple stain and thionin in 1 per cent solution (aqueous) were also employed, the two latter with the special end in view of determining, if possible, the nature of the granula found in many of the cells which were abundantly scattered and grouped throughout the tissues under consideration.

While the following description is based mainly upon the appearances found in the uvula obtained from the patient whose clinical history has already been cited, the five others which were obtained for comparative and corroborative examinations have also been carefully studied and will occasionally be referred to in what follows.

It seems desirable, before entering into the details of the degenerative process under consideration, to give a brief description of the tissues of the uvula in general.

The epithelial covering of all of the uvulæ (excepting the one from an infant) seemed to be slightly thickened; the anterior (oral) surfaces were corrugated, and in the one designated as "smokers' uvula" the corrugations were most marked. Posteriorly (pharyngeally) their surfaces were comparatively smooth, and the layer of epithelial cells was much thinner than that of the anterior surfaces (Plates XLII-XLIII). In both regions the nuclei of the epithelial cells presented in large number the various phases of typical mitotic activity. No ciliated epithelium was found. Careful examinations of many sections of the different uvulæ failed to disclose any evidences of either recent acute or chronic inflammatory changes in the epithelial covering (unless the number of mitotic nuclei or the seemingly slight thickening, already alluded to, should be looked upon as pathological), and in every instance the epithelial covering was intact.

General leucocytic invasion (phagocytosis) or accumulation of cells of this type in localized foci in the tissues of these uvulæ was never observed, though in all (save in the one from an infant) was found a form of granular cell which morphologically appeared to be identical with the Mastzelle of Ehrlich (Plate XLIX, Fig. 15). The granular cells failed, however, to give uniformly the decisive color reaction with the basic anilin dyes, which is a distinguishing characteristic of such cells. Once, in the formalin uvula, the granules of these cells reacted specifically, after staining with Nissl's methylene-blue sol., becoming purplish red, whilst the nucleus assumed a rather pale blue color. With thionin (which is recognized not only as an elective stain for mucin, but also as one for the granules alluded to above) there was no metachromatic reaction, granules and nuclei being stained alike and both very intensely. These cells were found in numbers in the lymph spaces of the connective tissue, the sheaths of the blood-vessels, the septa of the gland-lobules, the adipose tissue, and the perimysial tissue. As the result of pressure from surrounding parts, they presented varied shapes, the round and ovoid predominating, the nucleus being sometimes in the centre and sometimes at one end of the cell. As found between the muscle fibres, they were often elongated, the granules being disposed in a narrow band, gradually tapering to a point at either end.

Not infrequently there were found in the lymph channels of the delicate tunica propria lymphoid cells, which showed karyokinetic changes in several different phases, the most common being the monaster (mother star) and the well advanced dyaster (daughter star). These interesting histological phenomena were noticed only in the uvulæ fixed in mercuric bichloride solution, the most carefully instituted search in other uvulæ failing to reveal them. The sections showing these figures were those which had been stained in a 1 per cent solution of thionin in distilled water for a few minutes, decolorized in alcohol of various strengths and finally mounted in balsam. They are probably not abnormal appearances.

The lymph capillaries and spaces in both the tunica propria and the submucosa presented evidences of crowding with lymph cells, which at some points was more pronounced than at others. The presence of these lymphoid cells was particularly noticeable in the perivascular lymph sheaths as well as in the delicate connective tissue of the tunica propria which enters between the papillæ of the mucous membrane, and in a few instances actual lymph nodes were present in these regions. In the deeper layers of the submucosa the lymphoid cells were not so numerous.

Many of the granular cells already mentioned were found scattered throughout this latter tissue. Within many of the lobules of the mucous glands, with which the uvula is well supplied, lymphatic follicles were frequently observed in close apposition to the glands. These lymph nodes exhibited the characteristic denser cortical portion and the lighter central areas, corresponding to the germinal centres. The lymph channels going to and passing from such nodes could be easily traced in the intertubular connective tissue septa by the lymph cells which they contained. Such appearances are doubtless entirely normal.

All of the uvulæ were found to be richly supplied with blood-vessels, of which the larger were found in greater number at the periphery in the submucoid connective tissue, where they formed a network completely investing the uvula. The main and largest blood-vessels, which were few in number, pursued a central course, longitudinally, through the uvula, giving off branches at intervals, which again subdivided to form the capillary system of the various tissues situated in the interior of the organ. The tunica propria obtained its capillary blood supply from the peripheral blood-vessels already alluded to.

The blood-vessels, both large and small, as well as the capillaries, presented no noticeable abnormalities in any of the uvulæ. Ruptured blood-vessels, hæmorrhages or extravasations were never noticed. In the blood-vessels of the uvula fixed in formalin curious egg-shaped formations were noticed, of a clear homogeneous structure, which were arranged peripherally and seemed to be attached to the endothelial cells lining the vessel walls (Plate XLVI, Fig. 1).. The blood corpuscles in these vessels, as a rule, were crowded to the centre, filling up the rest of the lumen of the blood-vessel. This appearance was noticed not only in this uvula, but also in one fixed in alcohol, and undoubtedly represents an artefact, produced by the fixing agents employed.

The uvula is well supplied with glandular tissue. The mucous glands were found to occupy the upper third or proximal portion of most of the uvulæ examined (Plate XLII). The interacinous connective tissue contained single fibres of striated muscle, and in some instances bundles of four or five such fibres could be seen running between the lobules of the glands. This arrangement of the striated muscle fibres was particularly apparent in the uvula obtained from an infant at autopsy. Fat cells, in groups of from five to fifty or more, were also noticed interspersed in the connective tissue septa between the lobules of the glands, along with the gland ducts, many of which were filled with secretion.

In one of the uvulæ, which will be spoken of as "smokers' uvula," a most remarkable hyperplasia of the mucous glands was apparent, which could be seen to extend as a continuous chain from the point of abscission down to the tip or distal end of this uvula (Plate XLIII). In every instance, as is apparent in Plates XLII and XLIII, these glands occupied positions relatively nearer the posterior or pharyngeal surface than the anterior or oral. Most of the cells lining the tubuli of these glands exhibited a finely granular condition of their protoplasm. The uvula hardened in mercuric bichloride solution showed this granular condition in a most marked degree. In one of the sections made from a formalin preparation and stained in dahlia solution for twenty-four hours, well decolorized in alcohol and subsequently stained with methylene-blue, it was noticed that here and there isolated tubuli retained the dahlia stain with great pertinacity, the remaining tubuli having given up the latter stain and taken the blue quite electively.

Snarls and wisps of nerve tissue were found here and there cut in various directions, transversly, obliquely and longitudinally, and appeared to be normal in every respect. The myelin or medullary sheath of those found in the uvula fixed in mercuric bichloric solution appeared distinctly and coarsely granular, for which condition the hardening fluid used was undoubtedly responsible. Ganglionic nerve cells were not recognized in any of the uvulæ examined.

The general connective tissue of the uvula appeared to be more or less increased in all excepting the infant's uvula (autopsy), and was least marked in the one hardened in formalin. White fibrous connective tissue seemed to predominate throughout, the finer threads of the elastic variety being less common. These elastic fibres were easily demonstrable by a prolonged (over night) immersion in a .1 per cent solution of methyl violet, which stained them with precision and great intensity.

In the uvula hardened in alcohol (smokers' uvula) the hyperplasia of the connective tissue was much more advanced than in any of the others, and in many places its resemblance to scar tissue was very pronounced, it being but sparsely supplied with blood-vessels and almost entirely devoid of nuclei. van Gieson's picric-acid-fuchsin mixture stained it intensely.

CHANGES IN THE STRIATED MUSCULAR TISSUE.

At the point of abscission most of the muscular fibres showed very distinct, even exaggerated, transverse and longitudinal striations. The cross striations seemed coarser and the interfibrillar spaces wider than in a normal fibre, while the whole fibre presented an œdematous and swollen appearance (Plate XLVI, Fig. 2). Fibres of diminutive calibre at this point of the uvula might be looked upon as longitudinal peripheral segments of fibres which were only partially in the plane of the section. Here and there, but only at this part of the uvula and at their cut ends, fibres may be found which present somewhat the appearance of Zenker's waxy degeneration. As the resemblance to this form of degeneration was observed only here, it is probable that the surgical trauma inflicted in the removal of the uvula was responsible for its appearance, as Erb* and Weber† have demonstrated this experimentally with wounded muscle of living animals.

Plate XLVI, Fig. 2, shows two œdematous and swollen fibres with exaggeration of their cross and longitudinal striæ, and near the end of one of them a nuclear invasion which will be noticed further on under the heading "Nuclei of the Muscular Tissue." Following these fibres towards the distal or free end of the uvula, others were found in which peculiar absorptive and atrophic phenomena begin to manifest themselves, accompanied by what will be named (for want of a better designation) a peculiar bleb-like or vesicle-like homogeneous "deposit" at their peripheries (Plates XLIV and XLV), each vesicle, as a rule, containing a nucleus. A description of these contained nuclei will be given later.

In the beginning this deposited material may show only as a narrow, bright margin or line of a homogeneous nature along the periphery of the affected fibre, giving to the latter a double contour; or it may appear at irregular intervals, the sarcolemma being apparently raised here and there into small blebs or blisters containing the material referred to above (Plates XLVII, Fig. 7, and XLIX, Fig. 17).

At this stage of the alteration transverse striation is still distinctly visible, though not in so marked a degree as in those fibres which have been previously described. Longitudinal striation has been replaced by wavy and undulating lines corresponding to the individual fibrillæ which have now become twisted upon themselves, presenting an appearance not unlike the strands of a rope. This appearance is most noticeable at the free ends of those fibres which have advanced to this degree of the degeneration (Plate XLV). Some stress may be permitted in directing attention to this phenomenon of fibrillar twisting, in that it becomes, as a rule, apparent only after the marginal change begins to manifest itself.

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* Virchow's *Archiv*, xliii (1868), 108.

† Ibid., xxxix (1867), 216.

In some of the fibres, immediately at their periphery and between these and the vesicle-like structures, individual fibrillæ appear, which are breaking up into fine granules, yellowish in color, and not affected by stains, granules which under high amplification (× 1100) present the appearance of pigment (Plate XLVII, Fig. 6).

More or less opacity and a brownish yellow color are noticeable, in unstained sections, in all of the fibres which are well advanced in the degenerative process. Transverse cleavage into disks or blocks was noticed in only very few fibres, and then immediately in the neighborhood of the line of abscission. The same may be said of tortuous or kinked fibres, a few of which were found only at the point of surgical interference. van Gieson's picric-acid-fuchsin stained all fibres deeply and diffusely, as did eosin.

The final stage of the peculiar degenerative process is reached when a veritable cylindrical plug or mass composed of large blebs, containing small and large misshapen nuclei intermingled with pigmentary detritus, is formed, through the middle of which runs longitudinally a mere shadow, suggestive of the former site of a muscular fibre (Plates XLVII, Fig. 8, and XLVIII, Figs. 9 and 11). Occasionally such a mass may be found in which one, two, or more fibrillæ may still be distinctly visible with well preserved transverse striæ. Fibres were also observed which presented an unequal degree of degeneration, one end being only partially affected, whilst the other revealed the fibrillæ gradually being absorbed in a tapering fashion until they assumed a wedge shape, with a single projecting fibrilla, the whole being surrounded by cell-like vesicles containing nuclei (Plate XLIX, Fig. 13).

Examination of transverse sections of fibres shows in many a want of uniformity of distribution of the " deposited material ", that is, one-half only of a fibre may be involved, the other half remaining apparently normal (Plate XLVII, Fig. 5). Again one may see such a semi-degenerated fibre showing a few intact fibrillæ in the centre of the degenerated area.

In the transverse sections made from the uvula hardened in mercuric bichloride solution (Plate XLVIII, Fig. 12), a peculiar condition of the contents of the blebs was brought about by the action of this fixing agent; instead of the clear and homogeneous character of the material which was contained in the blebs of other uvulæ treated with formalin, Zenker's fluid, and alcohol, a granular precipitate, more or less fine, was noticeable, which in some of the cross-cuts of fibres was arranged radially, running from the remaining portion of the fibre in the centre of the bleb to what should represent the sarcolemma at the periphery. Subjected to a prolonged staining (24 hours) with Ehrlich's triple stain, the granular deposit took the fuchsin of the above mixture, the fibres, or what remained of them, taking both this and the orange with great intensity.

Although our material was hardly sufficient for the purpose, we have utilized it in order to fix the diameters of the muscular fibres with a view of determining whether hypertrophy existed. There is no literature extant indicating research on this particular point as regards the uvula. In an exhaustive and elaborate article by R. Mayeda,* an effort has been made to establish a scale of measurement for the different muscles of vertebrates. It is sufficient to state here that the conclusions arrived at are such that not only is it necessary to select muscles for comparative measurements which are anatomically identical and from the same species, but the conditions of nutrition and general health must also be taken into account in making such calculations.

Of the six uvulæ examined, from which the infantile uvula, on account of the primitive development of its muscle fibres, must be excluded, only one (the formalin specimen furnished by Dr. Gamble) could be used for the purpose of supplying a standard for making comparative measurements. This uvula may be considered as being practically normal with reference to all of its tissues, and its muscle fibres presented an average diameter of 0.019 to 0.023 mm. (Plate XLVI, Fig. 1). The uvula hardened in Zenker's fluid, with subsequent alcohol treatment, presented fibres which measured from 0.035 to 0.040 mm. (Plate XLVI, Fig. 2). The one hardened in merc. bichlor. sol. (Dr. Gamble) presented fibres which measured up to 0.050 mm. The peculiar muscular degeneration in this uvula was very marked, every fibre which appeared in the sections (all of which were transverse) showing it (Plate XLVIII, Fig. 12). The measurement of one of the largest of these fibres (already stated as 0.050 mm.) was made without including the peculiar marginal " deposit " already described. If the " deposit," which presumably is limited by the sarcolemma on the one side, and what represents the true muscle fibre be included, the measurement is brought up to 0.075 mm.

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* *Zeitschr. f. Biol.*, n. F. ix (1890), 119.

Nuclei of the Muscular Tissue.—Proliferation and polymorphism of the nuclei, characteristic of many forms of degeneration and of other lesions of muscle, are most prominent phenomena of the peculiar metamorphosis here under consideration. The shape, size and position of the nuclei, however, are so varied that a detailed description is necessary in order to give a comprehensive idea of them.

Fibres which are incipiently affected show proliferating nuclei of nearly normal shape, but somewhat enlarged; and as long as the nuclei proliferate unresisted and can preserve certain measured distances between themselves, they retain their normal shapes (Plate XLVI, Fig. 3). At such points, however, where they become crowded they assume flattened or irregular shapes and may leave their line of proliferation, either dipping down into and among the fibrillæ or becoming heaped up at one end of the fibre into an irregular mass (Plate XLVI, Fig. 2).

Noticeable also were nuclei flattened at their ends and hollowed out at their central portions, on either side, giving them an appearance which much resembled empty cotton spools. Plate XLVI, Fig. 4, presents this condition better than any description. Whether this long string of proliferated nuclei as depicted in this figure represents the dividing line between two fibres which are in juxtaposition, or whether they occupy a position in the centre of a single fibre, could not be determined by microscopic examination of the specimen, as the fibre was immediately lost in glandular tissue on the one hand and in areolar on the other, without giving satisfactory evidence as to this point.

Long, large nuclei, pointed at their ends with their bodies crenated or serrated, the serrations corresponding accurately to the transverse striæ of the fibre (if one imagines the striæ as projected through the narrow zone of homogeneous protoplasm which surrounds them), were frequently observed. These nuclei corresponded closely to the description given by Martius and Hansemann* of those found in the voluntary muscles in a case of myotonia congenita intermittens. The nuclei in our cases, however, were not so numerous, nor were they found in such long strings as in the case described by these writers. They were for the most part discretely scattered over fibres which still retained many of the attributes of normal fibres, such for instance as those which were situated high up in the proximal portion of the uvula.

The structure of the nuclei, with the exception of their crenated surfaces, was fairly well preserved, chromatin granules and the nucleoli being tinged almost perfectly by the proper stains.

Nuclei were frequently found with their long axes placed transversely to that of the fibre and with a tendency to become irregularly disposed.

The majority of the nuclei show, completely surrounding them, a more or less extended halo of a clear and homogeneous material, not affected by stains of any kind or combination (mercuric bichloride specimen excepted), and which seem to be limited by a very delicate and structureless membrane (Plate XLIX, Fig. 17). Fibres which begin to show this erratic disposition and shape of their nuclei are on the verge of manifesting the "bleb-like deposit" referred to or are already doing so. Along the periphery of such fibre nuclei may be seen around which the peculiar bullæ of "deposit" can be discerned in greater or less quantity. Occasionally an empty bleb may be seen, which may be accounted for by the plane of the section having been either above or below that of the nucleus (Plate XLIV).

As the atrophic and absorptive process in the fibres progresses, the blebs or vesicles increase in size, the nuclei retaining their position relatively to the centres of the blebs (Plates XLIV and XLV).

At a still later stage may be seen, in many of the blebs, in addition to the nucleus, a granular material, yellow in color, resembling pigment, which refused absolutely to stain with any dye, save the pigment found in the uvula fixed in merc. bichloride, and then only after a prolonged action of the staining fluid (Ehrlich-Biondi stain, 24 hours) (Plate XLV; Plate XLVIII, Fig. 9). With the gradual disappearance of the muscular fibre this pigmentary material augments in amount, depositing itself on and closely around the nucleus, which latter shows evidences of decreasing size and of irregularity of contour, with a tendency to fragmentation (Plates XLVII, Fig. 8; XLVIII, Fig. 9). At this stage one may observe here and there a vesicle or bleb in which its nuclear constituent has been entirely supplanted by pigmentary material, and in the final stage, when the fibre has been completely destroyed, its former site being occupied only by vesicles or blebs, the pigmentary deposit alone may mark the site of the original nucleus (Plate XLV). As a rule the pigment granules arrange themselves in two or three small clumps, irregularly in the centre of the bleb, though at other times the whole of the latter may appear to be peppered with this finely granular material. Now and again, however, the granules show a decided tendency to symmetry in arrangement, appearing as rosettes in some of the blebs and stellar-shaped in others. Their absolute indifference to stains serves to distinguish them from nuclear matter, the smallest portion of the latter usually staining deeply.

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* Virchow's *Archiv*, cxvii (1889), 587.

The nuclei now under consideration in connection with the advent of the pigmentary deposit are characterized by their irregular outlines, having for the most part lost their elongated oval form as well as all detail of structure, the great majority staining solidly and intensely. They appear sometimes as triangular bodies with sharp points; at other times as more or less rounded forms indented or honeycombed at their margins. Again, nuclei were observed shaped like blocks with sharp indentures at their peripheries, giving them a zig-zag or ragged appearance. Knobbed forms also came under observation. Here and there the nuclei failed to stain well, appearing of a pale azure with hæmatoxylin, showing in their interior one or two chromatic granules of a slightly darker shade.

Crescentic nuclei were frequently encountered enclosed in delicate vesicles of nearly spherical shape, the former appearing with their convexities closely applied to one segment of the vesicle and the whole body somewhat resembling in shape the crescent-shaped organisms found in the æstivo-autumnal type of malarial fever (Plate XLIX, Fig. 17). Nuclei of this shape were observed singly, in pairs and sometimes in greater numbers (five or six), arranged in a row or string, always at the periphery of a fibre, into which they seem to have sunken, and with their vesicular side turned outwards. Their occurrence is synchronous with the earlier stages of the degeneration. Optically, the delicate bleb or vesicle which encloses these nuclei seems to be of the same nature as that which constitutes blebs found elsewhere in the periphery of muscular fibres and already described.

Now and then a vesicle may be found which contains two or more irregularly shaped nuclei closely packed together; examination with high amplifications (800-1100) favors the probability that this appearance is due to the coalescence of two or more blebs. Plate XLIX, Fig. 14, illustrates this appearance, the centre of the field being occupied by a large apparently single bleb containing five deeply stained nuclei.

Evidences of regeneration of the muscle fibres of the uvulae were never observed. Such interesting histological phenomena, however, were kept in mind during the careful examination of some hundreds of slides, and it seems, in the absence of such regenerative efforts, that the muscular degeneration which has been described may be regarded as the cause of the prolapse and elongation of the uvula. The great hyperplasia of the mucous glands found in the specimen designated "smokers' uvula" in the text and shown on Plate XLIII, is probably analogous to similar glandular hyperplasiæ which occur in the pharyngeal mucous membrane in certain chronic inflammatory states.

The presence of muscular fibres within the glandular lobules suggests that they may exert an expulsive influence over the secretory contents of these glands.

I desire to direct special attention to the peculiar character of the changes in the striped muscle of the uvulæ described in this article and clearly demonstrated in the photographs. I have not been able to learn that identical changes have hitherto been described. As appears from the description which has been given, I regard these changes, although they are associated with marked nuclear proliferation, as essentially degenerative in character, their final stage being a nearly or quite total disappearance of the contractile substance of the affected muscular fibre. The frequency, the anatomical and physiological significance, and the satisfactory interpretation of these muscular alterations can be fully determined only by further investigation of a larger and more varied material.

The technique and apparatus employed in the production of the photographs with which this paper is illustrated have been fully described in the *Bulletin of the Johns Hopkins Hospital,* Nos. 62-63, May and June, 1896.

In conclusion, obligations are here acknowledged to Drs. W. H. Welch and L. F. Barker for their interest and for valuable suggestions during the course of this investigation.

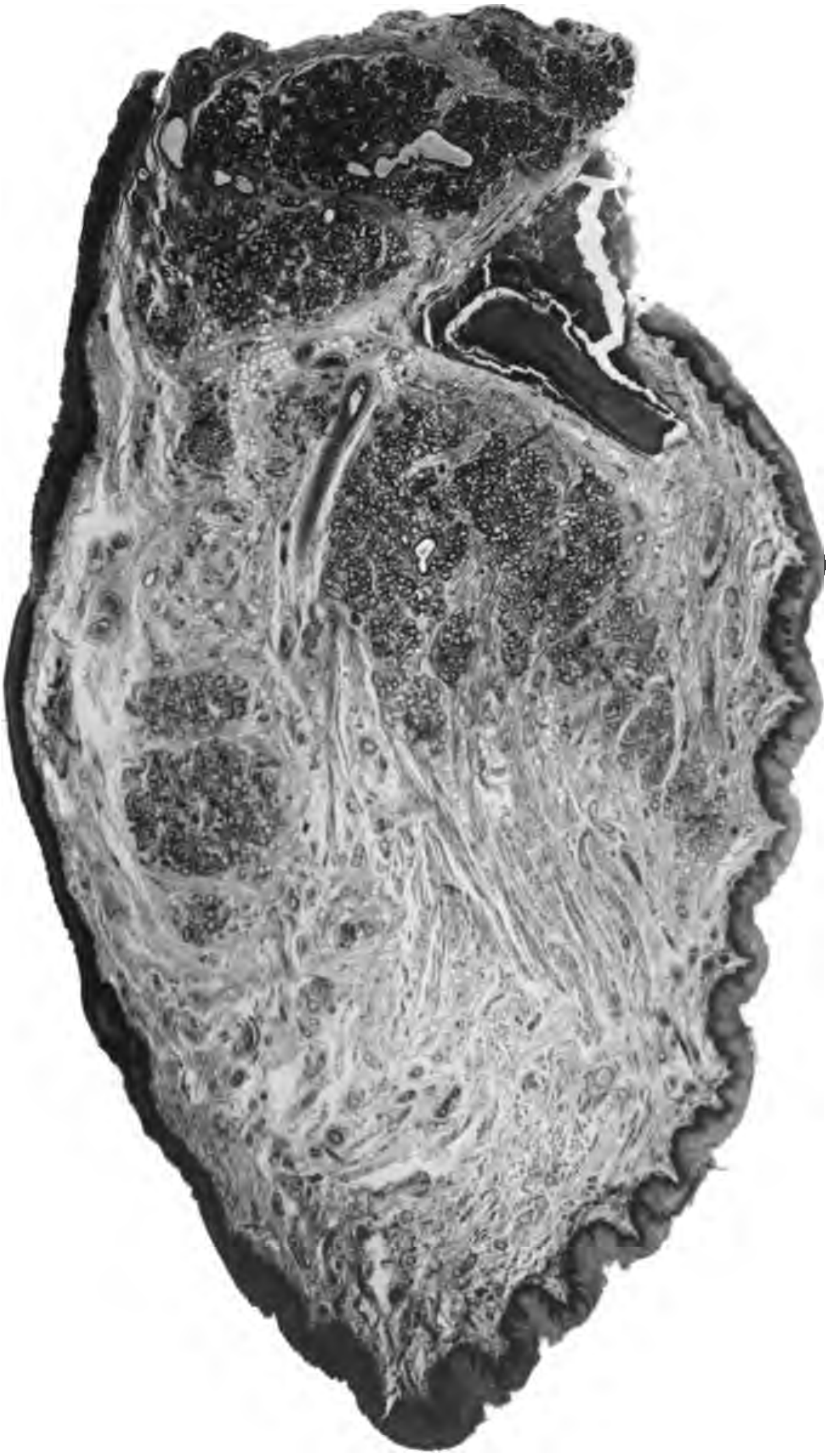
DESCRIPTION OF PLATES XLII-L.

PLATE XLII.

This photograph shows a longitudinal section (sagittal) at about the middle of the entire removed portion of the uvula. The thicker and corrugated mucous membrane to the right represents the anterior surface. The mucous glands occupy, for the most part, the upper or proximal portion of the uvula, although a few scattered patches may be seen lower down. The uvula is well supplied with blood-vessels, the greater number being found near the surface in the areolar tissue just beneath the mucous membrane.

The muscle fibres may be seen as narrow bands in the middle portion of the section, radiating in various directions, the anterior surface receiving a greater number than the posterior. The connective tissue, which was abundant, consisted of dense white fibres, arranged in fasciculi, its greater development being in the region of blood-vessels, around and between the lobules of the mucous glands.

Zenker's fluid preparation. Amplification = Zeiss 70 mm. = × 17½.



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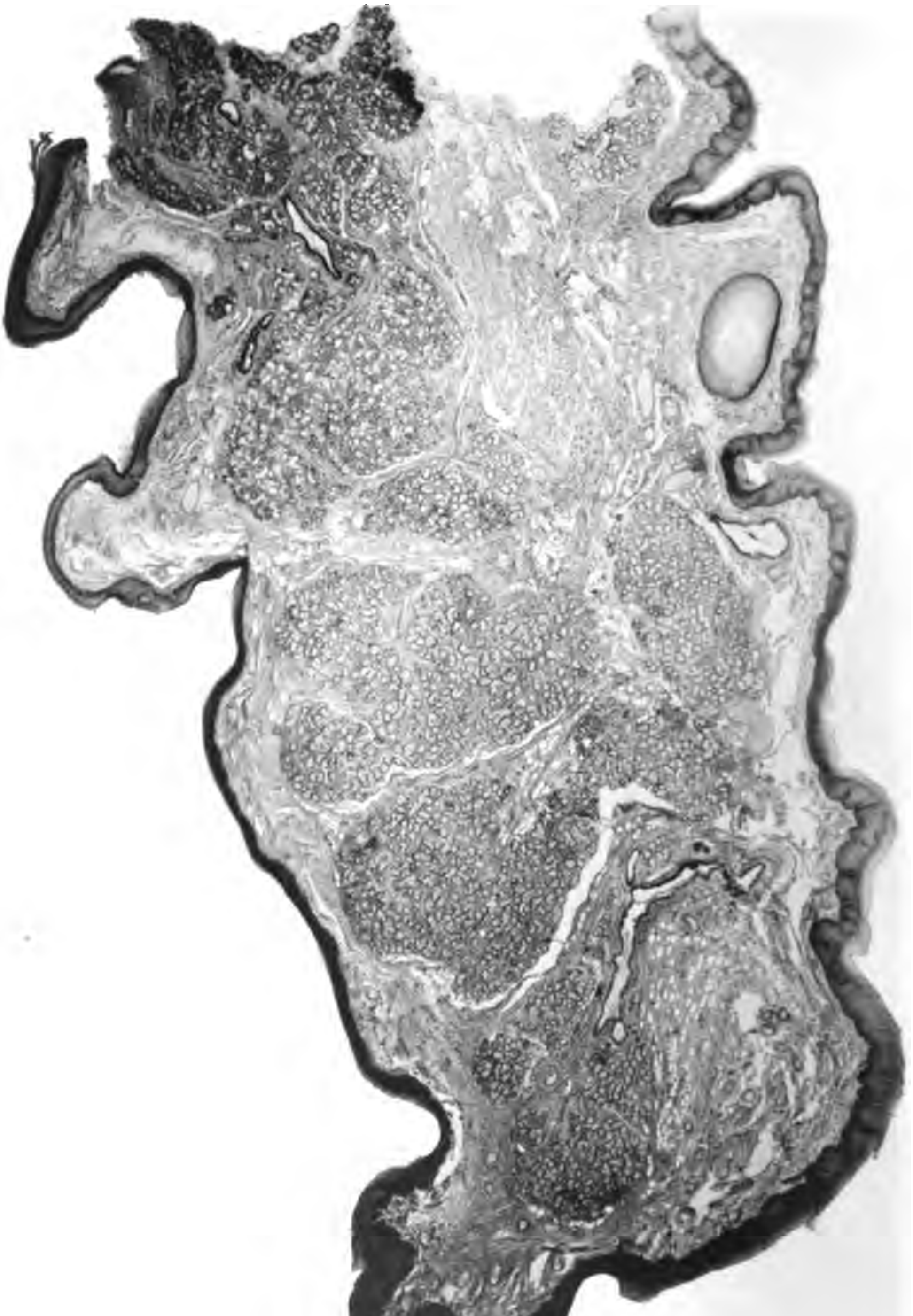

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PLATE XLIII.

"Smokers' uvula." Sagittal section through about the middle. There is great hyperplasia of the mucous glands, which may be seen as a continuous chain extending from the line of ablation to the tip of the uvula. Muscle fibres have entirely disappeared from the lower two-thirds of this uvula, being replaced by dense bands of connective tissue which ramify around and between the gland lobules, anastomosing at many points. The tissue is very sparsely nucleated and in some places approaches scar tissue in appearance. At the point of abscission a few muscle fibres were found, which, under a high power, did not appear to deviate much from the normal, a slight nuclear proliferation being the only departure. The irregular outline of this uvula is probably due in part to contractions caused by the connective tissue and in part to the action of the alcohol used in hardening. The surface to the left is the posterior or pharyngeal, that to the right the anterior or oral aspect of the uvula. The gland ducts are dilated, and the anterior epithelial covering is somewhat hypertrophied.

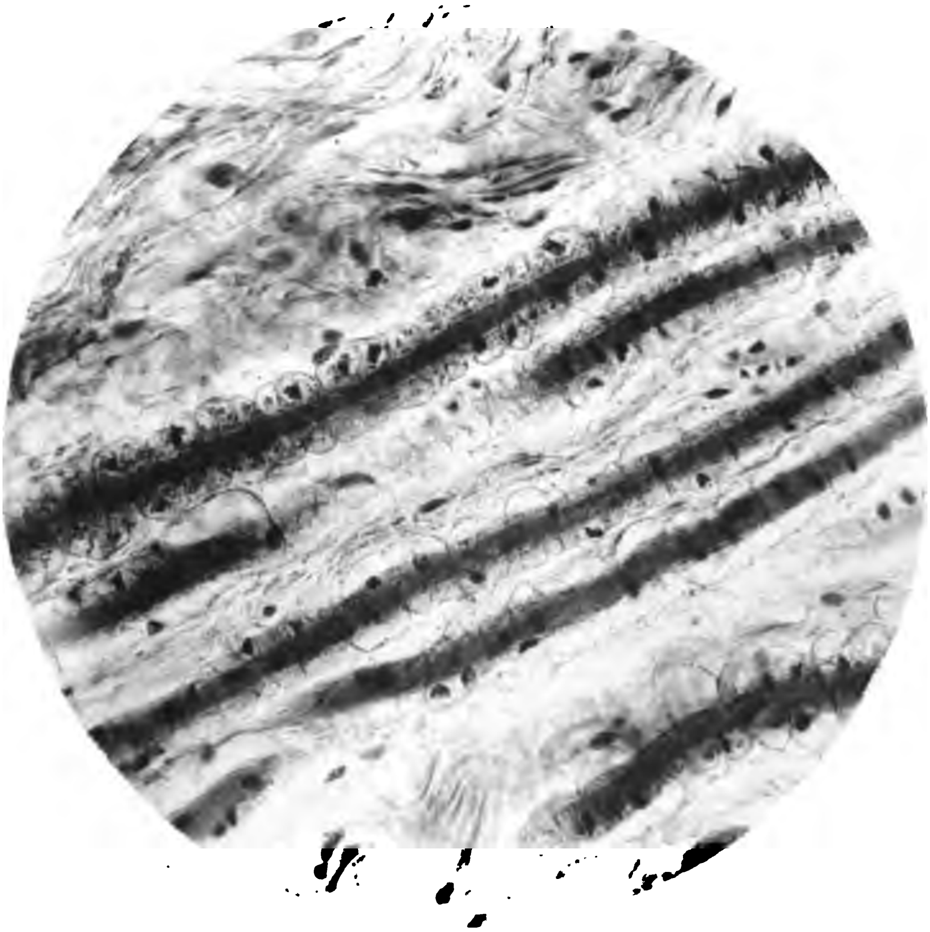
Amplification = Zeiss 70 mm. = × 17½.

PLATE XLIV.

This photograph shows five muscular fibres, parallel to each other, which exhibit the vesicular or bleb-like process typically, as well as the granular disintegration of some of the individual fibrillæ. Various phases of nuclear change are also well exhibited together with the distribution of granular pigment. For a more detailed description see text.

Amplification = Zeiss 4 mm. Apochromatic, N. A. 95. Compensat. proj. ocular No. 4. Camera length = 550 mm. = × 550.

THE JOURNAL OF EXPERIMENTAL MEDICINE. VOL. III. PLATE XLIV.



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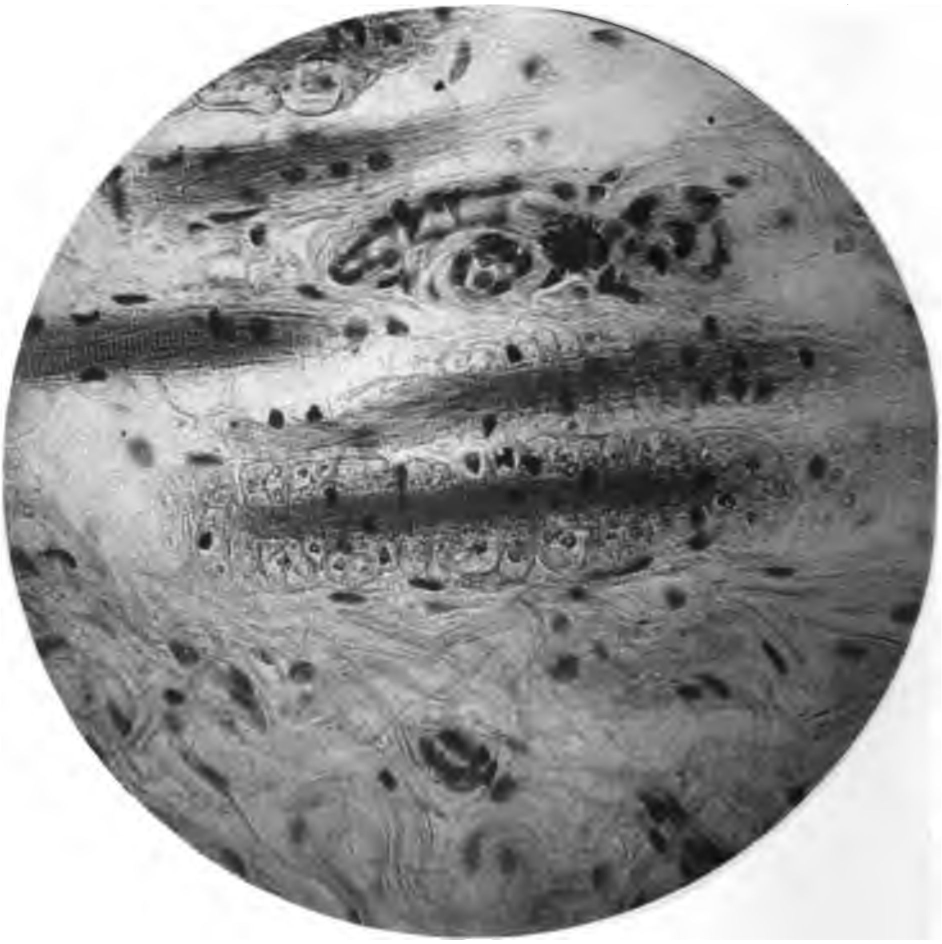

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PLATE XLV.

Four muscle fibres show advanced degeneration. The twisted condition of the fibrillæ is prominent, the transverse striæ remaining clearly defined. Many blebs surrounding the fibre in the centre of the field contain pigmentary deposit in lieu of nuclei.

In the lower segment of the field wavy connective tissue is apparent, its cells sending out long ramifying processes. It stained deeply with van Gieson's picric acid and fuchsin solution.

Amplification = Gundlach achromat. ⅙. Project. ocular No. 4. Camera length 550 mm.

PLATE XLVI.

Fig. 1. This figure shows in the lower half of the field four or five muscle fibres, which are almost normal save that the individual fibrillæ show a slight tendency to disarrangement. It is difficult to clearly differentiate, excepting at one or two points, the sarcoplasmic, endomysial and endothelial nuclei. The clear space in this specimen is a fat cell; just above this there is an arteriole in transverse section containing clear oval bodies, probably artefacts, which are closely attached to the inner wall of the vessel; they were observed in every blood-vessel of this uvula and in the one fixed in alcohol (smokers' uvula), but in none of the others. This uvula was fixed in 5 per cent formalin with subsequent hardening in alcohol. The muscle fibres measured from 0.019 to 0.22 mm.

Amplification = Gundlach achrom. 1/6. Zeiss project. ocular No. 4. Camera length 550 mm.

Fig. 2 shows two fibres swollen and œdematous; in a portion of the one at the left part of the field an aggregation of deformed nuclei may be discerned packed closely together. In the same fibre and opposite the nuclei, one or two wavy fibrillæ may be seen slightly lifted away from the rest, a tendency to a general disarrangement of the latter being manifest in both fibres shown. The transverse striæ are sharply defined. These fibres measured respectively 0.035 and 0.039 mm. in diameter. Stained with gentian violet in anilin water—xylol-anilin, xylol, balsam.

Amplification = Gundlach achromat. 1/6. Zeiss project. ocular No. 4. Camera length 550 mm.

Fig. 3. The muscle fibre here shown approaches the normal, a nuclear proliferation at either end and a slight increase in calibre marking the departure. This is the inaugural stage of the degeneration. With a little care, not only the ordinary transverse striæ, but also the intermediary, secondary, or "Dobie's lines" (in the light discs) may be distinctly made out. The large dark mass seen on the fibre is a precipitate from the fixing fluid used (Zenker's). The dark masses to the right are portions of the mucous glands in cross sections.

Amplification = Gundlach achromat. 1/6. Zeiss project. ocular No. 4. Camera length 550 mm.

Fig. 4 shows a row of proliferating nuclei; in shape they resemble empty cotton spools. This form is especially apparent in five or six of the nuclei situated in the middle of the string where they are in perfect focus. The clear space to the left of the fibre is a vein cut rather obliquely. The rest of the field is occupied by mucous glands.

Amplification = Gundlach achromat. 1/6. Zeiss project. ocular No. 4. Camera length 550 mm.

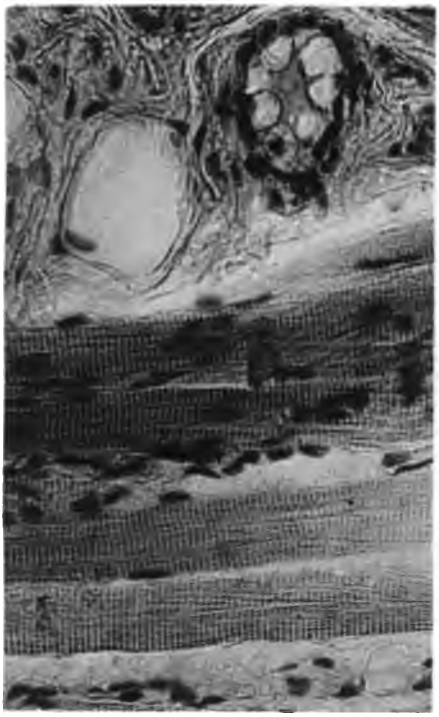

FIG. 1.

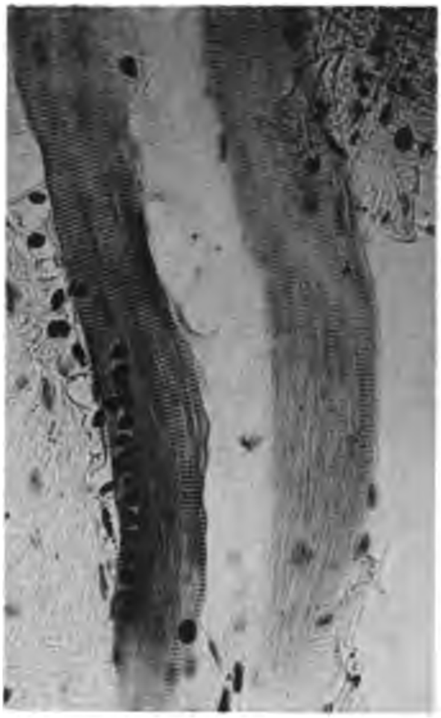

FIG. 2.

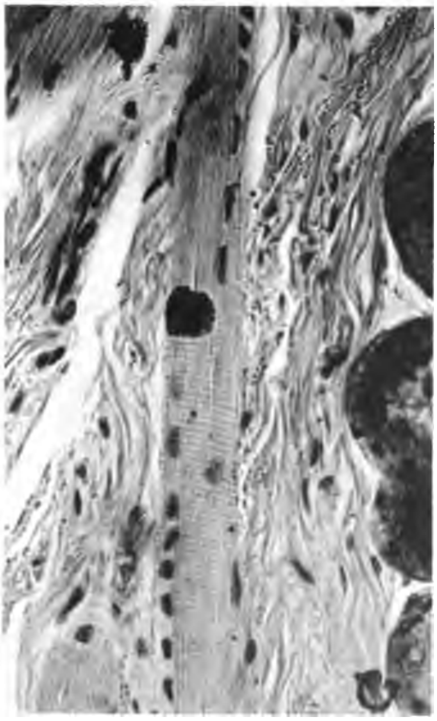

FIG. 3.

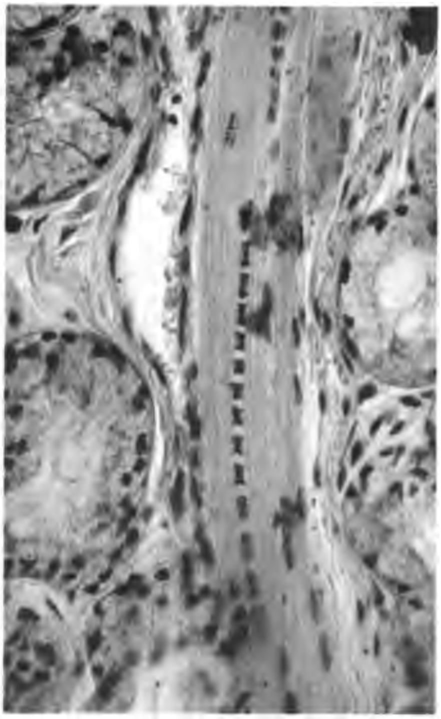

FIG. 4.

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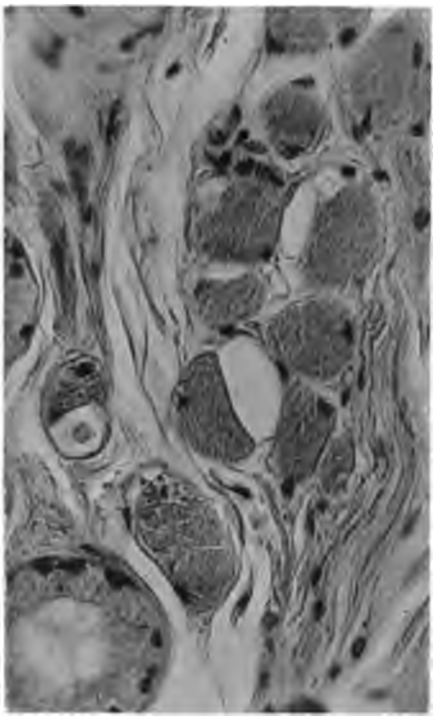

FIG. 5.

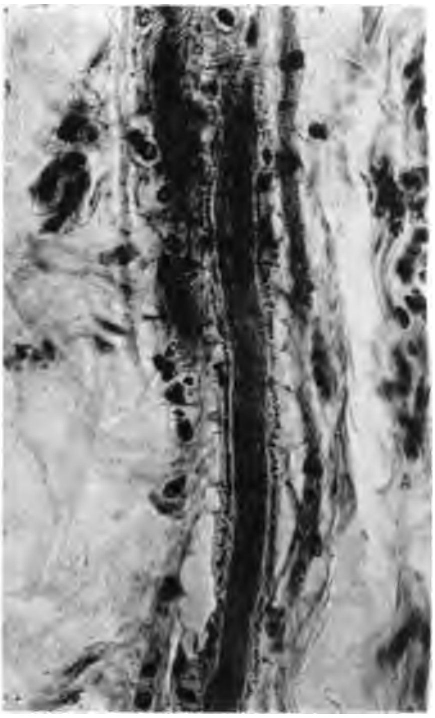

FIG. 6.

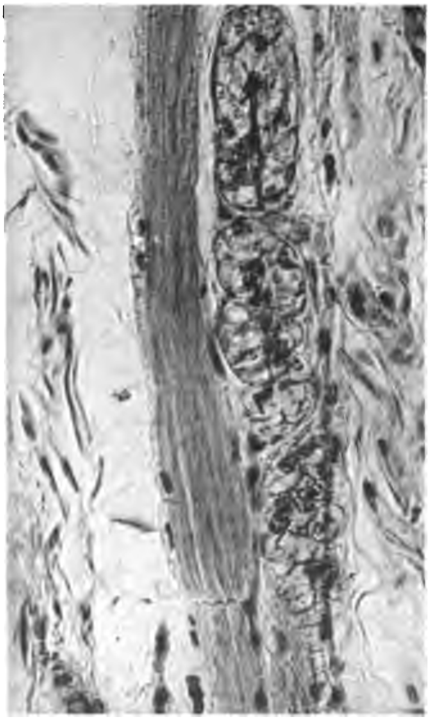

FIG. 7.

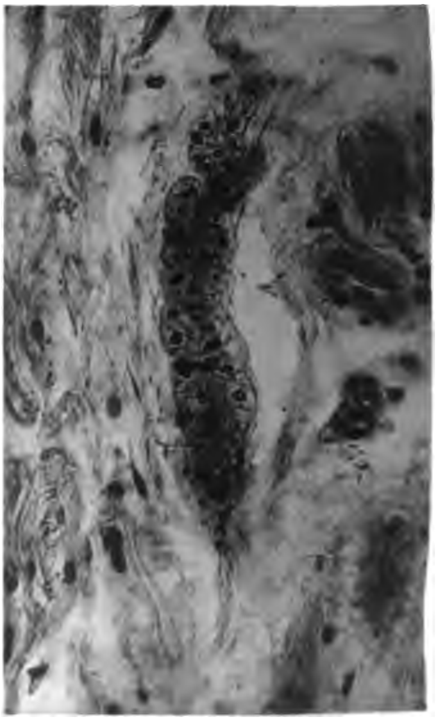

FIG. 8.

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Plate XLVII.

Fig. 5 shows a number of muscle fibres in cross section. The isolated fibre, to the left of the centre, shows the semi-degenerated condition mentioned in the text. The clear homogeneous material, with a few remaining fibrillæ in its centre, occupies one-half of the fibre. A nucleus is seen within the fibrillæ of the remaining half. Diagonally below this a large fibre appears, the fibrillæ of the upper portion of which are disarranged and coarse. Part of a nucleus is visible within them. " Cohnheim's fields " come clearly to view in the rest of the fibre. At the centre of the field a half-moon-shaped fibre may be noticed, closely adhering to which is a perfectly clear and homogeneous vesicular body of similar shape and size to the fibre with which it is associated. Like bodies, though smaller, may be seen in connection with other fibres in the field. Between two fibres, near the upper margin of the figure, a nuclear proliferation in the perimysium internum has taken place.

Amplification = Gundlach achromat. ⅕. Zeiss project. ocular No. 4. Camera length 550 mm.

Fig. 6 shows a fibre from either side of which a fibrilla has been separated which is breaking up into fine granules, seemingly in accordance with the lines of the transverse striæ. Immediately to the left of this fibre is a fibre the middle portion of which has apparently been totally destroyed, containing a few fragmentary nuclei (karyorhexis) which mark its former site.

Amplification = Gundlach achrom. ⅕. Zeiss project. ocular No. 4. Camera length 550 mm.

Fig. 7 shows two muscle fibres which extend entirely through the centre of the field. The one to the right has been completely metamorphosed into a mass of blebs; in the extreme upper part of this mass and in its middle a short, thick and dark line may be seen, which represents the last few remaining fibrillæ. The fibre to the left shows constrictions at intervals throughout its length (which may also be noticed on the other one), but it still retains many of its normal attributes. The constrictions are due apparently to delicate connective tissue processes which encompass the fibres at the points mentioned.

Amplification = Gundlach achrom. ⅕. Zeiss project. ocular No. 4. Camera length 550 mm.

Fig. 8. In the centre of the field is a cylindrical mass composed wholly of blebs; some of these contain nuclei of varying shape and size, several of which are interesting in that they present, if carefully examined, appearances somewhat like the " monaster " stage of dividing nuclei. Some blebs contain pigmentary deposit only, while in others there is also nuclear material. The darkened middle portion of the mass, seen indistinctly through the blebs and extending the entire length, marks what is left of the degenerated muscle fibre.

Amplification = Gundlach achrom. ⅕. Zeiss project. ocular No. 4. Camera length 550 mm.

PLATE XLVIII.

Fig. 9. Near the centre of the field is a mass of blebs containing nuclei and pigmentary material; the dark central shadow in this mass represents what remains of the muscle fibre. At the upper end of the mass are several blebs which contain pigmentary material only, the nuclei having disappeared. Above is a blood-vessel in transverse section. To the right are portions of fibres in various stages of degeneration.

Amplification = Gundlach achrom. 1/8. Zeiss project. ocular No. 4. Camera length 500 mm.

Fig. 10 shows a section of the uvula described as "smokers' uvula" in the text, and is introduced to show the very dense nature of the connective tissue, which in connection with glandular hyperplasia represented very nearly all of the tissues of which this uvula was composed. This tissue contained few cells of any kind (fat cells excepted), and its vascular supply was also small. The fat cells are represented by the clear spaces.

Amplification = Gundlach achrom. 1/8. Zeiss project. ocular No. 4. Camera length 550 mm.

Fig. 11. In the centre of the field a fibre is shown which has been almost completely metamorphosed into blebs; a narrow dark band running through the middle of the mass indicates the remains of the muscle fibre. The clear space partially shown in this field is a portion of a fat cell.

Amplification = Gundlach achrom. 1/8. Zeiss project. ocular No. 4. Camera length 550 mm.

Fig. 12 is a transverse section of the muscle fibres from the specimen hardened in mercuric chloride solution. The staining (Ehrlich-Biondi, 24 hours) was unfortunately photographically incorrect, the negative being very thin and without contrast. The resulting picture, whilst showing these defects, nevertheless brings out the peculiar action of the bichloride salt on the material of the degeneration around the muscle fibres. The granular condition of the blebs is well shown, as is the radial arrangement, more minutely described in the text.

Amplification = Gundlach 1/8. Project. ocular No. 4. Camera length 550 mm.

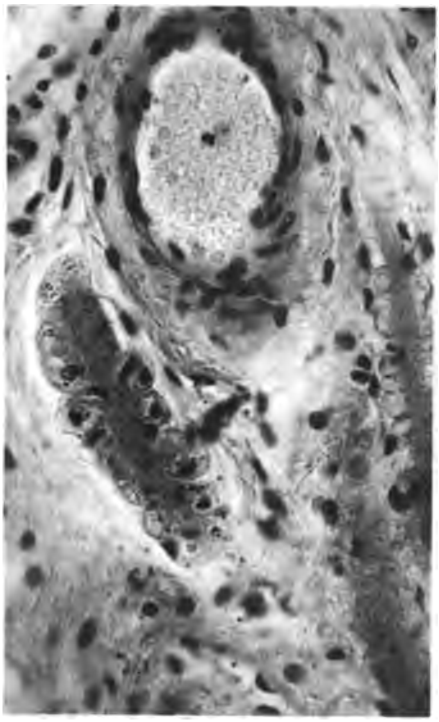

FIG. 9.

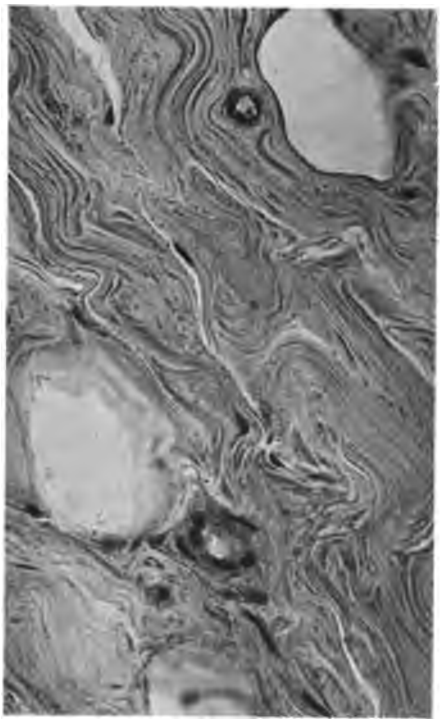

FIG. 10.

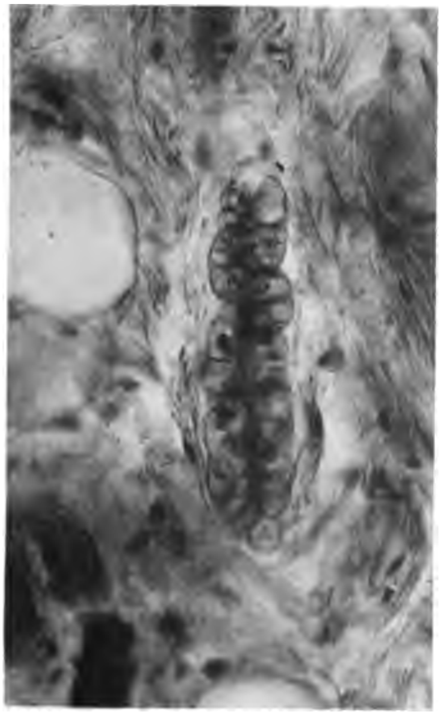

FIG. 11.

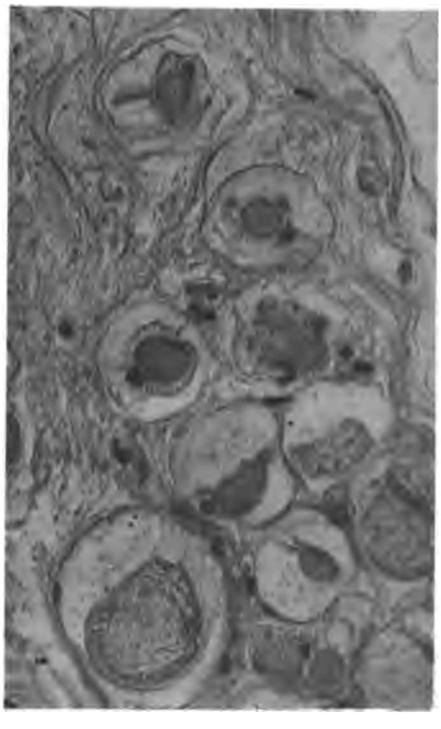

FIG. 12.

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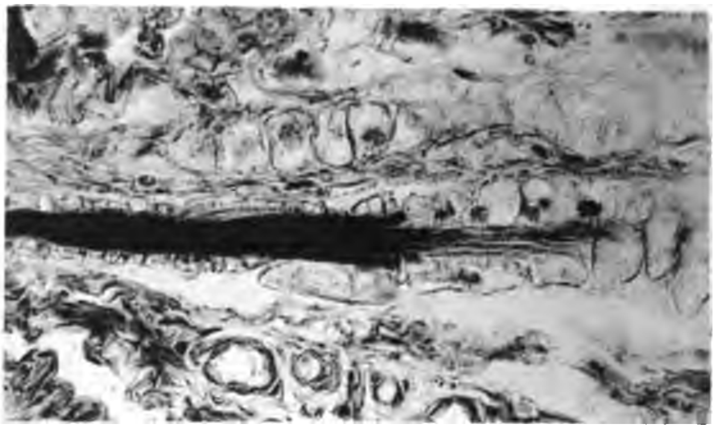

FIG. 13.

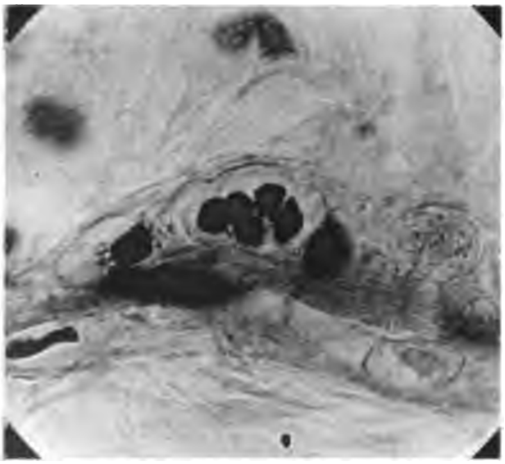

FIG. 14.

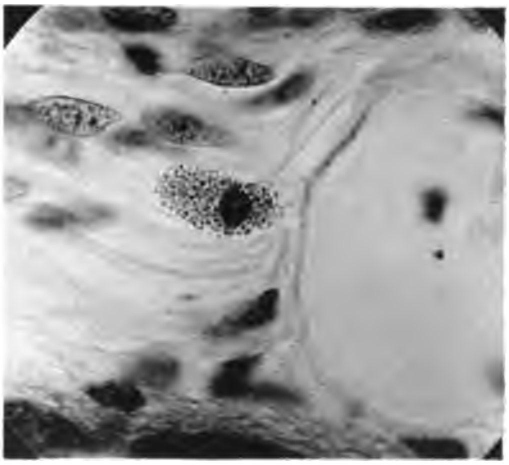

FIG. 15.

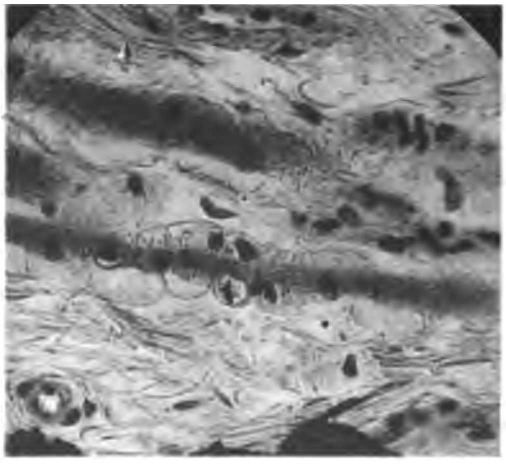

FIG. 16.

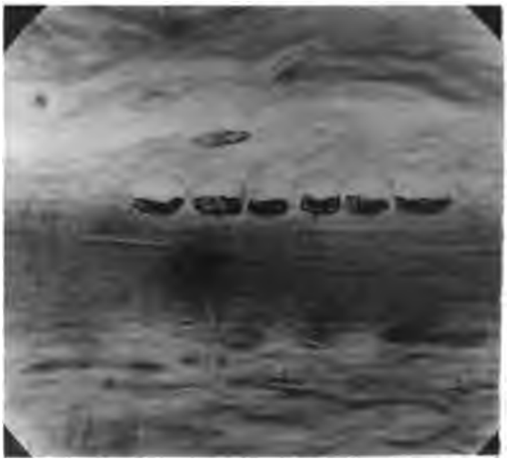

FIG. 17.

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PLATE XLIX.

Fig. 13. Depicting a muscle fibre, one end of which shows the degenerative process in a most striking manner. The uvula from which this slide was prepared was hardened in a saturated (aqueous) solution of mercuric chloride and was tinged with the Ehrlich-Biondi mixture. The blebs are shown as separate and irregularly shaped masses with distinct outlines, some with and others without nuclei. Five or six fibrillæ are seen projecting from the body of the darkened fibre which have preserved their transverse markings excellently.

Amplification = Gundlach achrom. ⅕. Zeiss project. ocular No. 4. Camera length 550 mm.

Fig. 14 shows an atrophic fibre surrounded by large blebs or vesicles. In the centre of the field is a single large bleb containing five irregularly shaped nuclei, intensely stained. Immediately to the left of this another may be seen containing a single nucleus, with pigmentary material deposited around it. Near the left margin of the field and below the fibre is an elongated and clavicular-shaped nucleus. On either side of the fibre to the right are blebs devoid of nuclei, containing pigmentary deposit, which in one instance has been evenly disseminated throughout the whole of a bleb, whilst in the other it appears massed in the centre.

Amplification = Zeiss oil immersion. 2 mm. achrom. N. A. 130. Proj. ocular No. 4. ×1100.

Fig. 15 shows a cell the protoplasm of which is studded with granules. Cells of this type were abundantly distributed throughout the connective tissue, in lymph spaces, and between the muscle fibres (perimysium internum). The granules differed as to size, some being as fine as dust, others coarse, the latter being shown in the cell in the centre of the field. The spindle-shaped nuclei with sharply stained nucleoli present in the rest of the field are the fixed nuclei of the connective tissue, the wavy fibres of which are evident. The clear round space to the right is a fat cell. The staining reactions of the granula of these cells have been considered in the text.

Amplification = Zeiss oil immersion, achrom. 2 mm. N. A. 130. Project. ocular No. 4. Camera length 550 mm. ×1100.

Fig. 16. This photograph is introduced specially for the purpose of demonstrating the indiscriminate distribution of the pigment granules noticed in some of the fibres. By examining the photograph under an ordinary hand-lens, the material may be seen to consist of dust-fine granules, sown over and throughout the entire course of the muscle fibres, at some points more thickly than at others, irrespective of blebs or nuclei. Other characteristics have been fully described in the text and in the explanatory pages accompanying the plates.

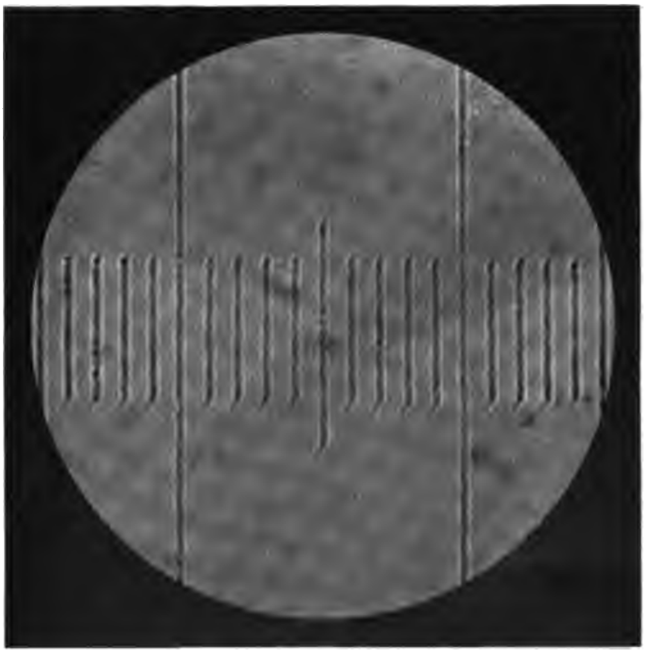
Amplification = Gundlach achromat. ⅕. Project. ocular (Zeiss) No. 4. Camera length 550 mm. ×400.

Fig. 17 shows a muscle fibre with a string of six crescentic nuclei attached to its upper border. Each nucleus is enclosed in a delicate, more or less spherical vesicle. The position of the nuclei is such that their convex surfaces seem to be united to a proportionate segment of the vesicle, the latter being distinctly separated from one another. The nuclei present irregular outlines at their free borders, and appear to be partly imbedded in the substance of the fibre. The finer details of the muscle fibre were sacrificed to those of the nuclei and vesicles, it being found impossible to bring both into the same optical plane with the high magnification employed.

Amplification = Zeiss oil immersion. 2 mm. N. A. achromatic. Project. ocular No. 4. Camera length 550 mm. 130 × 1100.

Plate L

Represents a millimetre scale, divided into 0.01. The photo-micrograph was obtained by the combined use of a 1/6 achromatic objective made by Gundlach, Zeiss' projection ocular No. 4 (non-compensative) and a camera length of 550 mm., producing an amplification of ×400, with which combination most of the photo-micrographs illustrating this article were made. When other amplifications have been employed for special purposes, they have been duly noticed in the explanatory pages accompanying the plates.



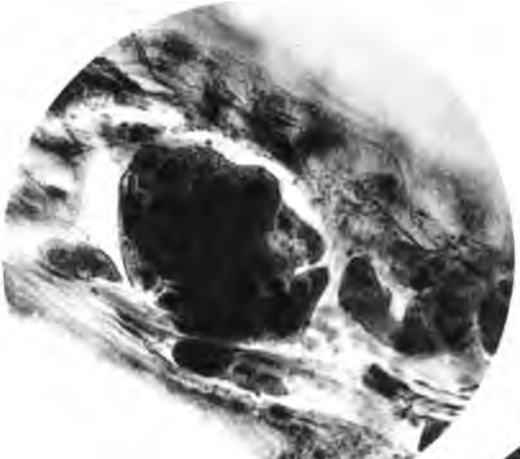


FIG. 1.

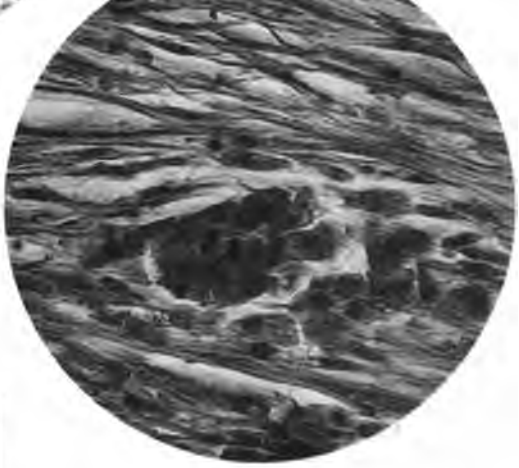


FIG. 2.

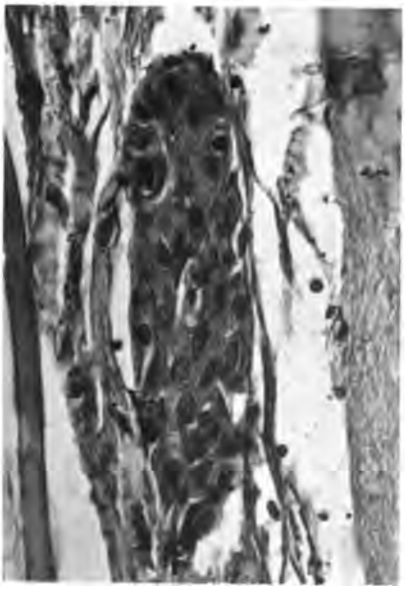


FIG. 3.

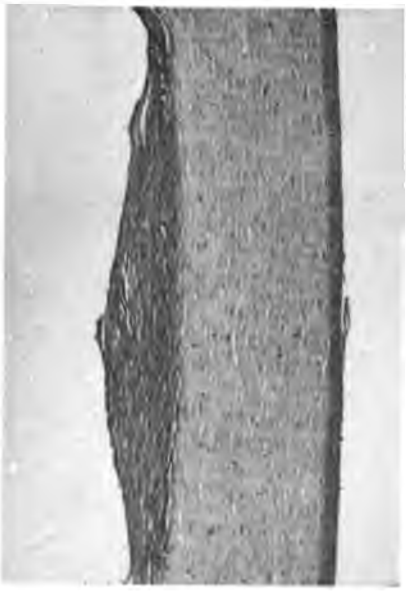


FIG. 4.

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THE FATE OF THE GIANT CELLS WHICH FORM IN THE ABSORPTION OF COAGULATED BLOOD SERUM IN THE ANTERIOR CHAMBER OF THE RABBIT'S EYE.

By LUDVIG HEKTOEN.

PLATE LI.

In this article it is the purpose to describe briefly the results of some experiments which show that the multinucleated giant cells which form during the absorption of foreign bodies in the tissues may divide into viable cells.

Concerning the extensive literature about giant cells—their formation, function and fate—reference is made to the article in this Journal by the writer entitled " The Fate of Giant Cells in Healing Tuberculous Tissue as observed in a Case of Healing Tuberculous Meningitis,"* in which it is pointed out that giant cells in tuberculous tissue, under certain conditions, undergo progressive changes and separate into small living cells.

THE EXPERIMENTS.

Small pieces of sterile, solidified blood serum, such as is ordinarily used as a culture medium, were inserted under aseptic precautions and cocaine anæsthesia into the anterior chambers of several healthy rabbits' eyes through a small incision in the sclera near the corneal margin. Once a bit of sterile potato was introduced. These operations were skilfully made for me by Dr. Cassius D. Westcott, Instructor in Ophthalmology in Rush Medical College, and I wish to thank him for his kind aid.

After perfecting the technique the operations were followed by but little reaction; in some instances the vessels in the sclera were congested for three or four days, the cornea rather cloudy, the anterior chamber turbid, and more or less photophobia was apparent. Soon after their implantation a small greyish white rim would become visible around the foreign bodies, which gradually disappeared until, if allowed, only a small, greyish, semi-opaque thickening in the inner surface of the cornea remained. In a few cases the blood serum was gradually dissolved without any accumulation taking place around it, the cornea and anterior chamber remaining perfectly clear. In the case of the bit of potato and when pieces of dry and hard serum were used, the absorption always proceeded slowly and with well-marked anatomical changes.

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* *The Journal of Experimental Medicine*, iii (1898), 21.

The animals were killed at intervals of from a few days to six or more weeks after the operation. The eyes were fixed in corrosive sublimate and the piece of cornea and iris, including the site of the foreign body, embedded in paraffin, cut serially, fixed on the slide and stained with hematoxylin and eosin, van Gieson's stain, etc.

THE HISTOLOGICAL EXAMINATION.

Comparatively slight attention was given the study of the early changes. Suffice it to say that the emigration of leucocytes from the vessels of the iris took place to a limited extent only, at least in the cases in which bits of solid blood serum are concerned. The leucocytes present were of the polymorphonuclear variety, and they appeared to become loaded early with granules of serum, after which some of them returned to the iris, in which a few collections of such cells were found. In the later stages of the resorptive process leucocytes were not observed.

The new cells produced come almost wholly, it would seem, from the layer of polyhedral cells clothing the inner surface of the posterior elastic lamina (Descemet's membrane) and the anterior surface of the iris. The posterior lamina itself generally remains quite intact. The new cells, which are round, oval or polyhedral, accumulate around the foreign body and penetrate into its interior, generally breaking it up, so that there is formed a mass of cells which adheres to the elastic layer and encloses the granular detritus of the coagulated serum. Most of the cells take up granules into the interior of their cytoplasm, often to such an extent that the nucleus and the entire cell appear as if buried beneath the granular mass. The serum granules, whether free or intracellular, are easily recognizable because of their brownish or golden yellow color (derivatives of hæmoglobin pigment).

During this time there are formed a small number of multinucleated giant cells or plasmodial masses, which appear, as far as these observations show, to result from fusion of many small cells. These giant cells do not differ in form and in their general characteristics from those ordinarily observed in connection with the absorption of sterile foreign bodies elsewhere. Their size and the number of nuclei, which are scattered uniformly throughout the body of the cell, vary considerably. In the earlier stages the cells are loaded with serum granules and are of circular outline (Plate LI, Fig. 1); later when the foreign body has been largely removed the giant cells become oval or oblong, due, it would seem, to the pressure of the new fibres that in the meantime have formed around them (Plate LI, Fig. 3).

The granules of solid blood serum without and within the cells gradually disappear, largely, it would seem, by means of a process of intracellular digestion or solution; in some of the giant cells as well as in the smaller cells occur vacuoles in the cytoplasm containing granules; some of the serum, but not much, is also transported into the iris by leucocytes.

Simultaneously with the destruction or removal of the foreign granules the uninuclear and multinuclear cells begin to show further and progressive changes that ultimately lead to the formation of a layer of tissue which much resembles the cornea in structure. These changes begin first and are farthest advanced at the periphery of the cell mass, extending little by little throughout the central parts.

The changes consist in the production of a fibrillated intercellular substance, or stroma, while at the same time the cells become spindle-shaped and elongated, the nuclei more slender and fusiform. All the stages in this transformation of embryonal cells to mature tissue, during which the granules of serum are destroyed, can be quite readily traced. In the later stages the fibrillæ of the new stroma are gathered together into interlacing bundles of considerable thickness (Plate LI, Figs. 2 and 3), the general course of which is parallel with that of the corneal lamellæ, from which they are separated by the intact posterior elastic lamina. While the course and arrangement of these new fibres are identical with those of the cornea, yet the new lamellæ are not so thick and densely interwoven as those in the cornea and they stain more deeply with eosin. This new tissue, the free surface of which becomes covered by a distinct endothelial lining, may finally appear as a mere rounded or pyramidal-shaped thickening on the posterior surface of Descemet's membrane (Plate LI, Fig. 4), or it may unite the cornea with the iris, especially if the foreign body found a resting place in the irido-corneal angle.

Lying in the spaces of the new tissue, sometimes apparently upon its fibres, are small cells like connective tissue cells, occasionally cells containing granules as well as free heaps of serum detritus and, in the stages not too far advanced, multinucleated giant cells in the process of separation into uninuclear cells.

It has already been stated that in the earlier stages the giant cells are more or less circular and the margins smooth and even. As the fibrillated stroma forms about them they may in many cases become oval and even distinctly oblong (Plate LI, Fig. 3), simultaneously with the disappearance of the serum, the long diameter corresponding to the direction of the fibres of the stroma. At this time they are usually entirely, or almost entirely, free from granules of serum; occasionally there may be vacuoles containing granules in the protoplasm. It should be emphasized that there are no signs of degeneration in the giant cells; the cytoplasm is reddish when stained by eosin and sometimes finely granular, but often it appears dense and homogeneous; the nuclei stain perfectly, and in one instance a dividing nucleus in the daughter star stage was observed in a giant cell.

As the serum disappears more or less distinct lines of cleavage become visible in the giant cells, at first most marked at the periphery; the margin is not smooth and even, but is fringed by the spindle-shaped projections of partly separated cells which seem to have a tendency to arrange themselves parallel with the fibres of the stroma (Plate LI, Fig. 3). Single uninuclear cells exactly similar to these partly detached masses are occasionally found about the giant cells. In some giant cells, especially those encountered in the older and firmer parts, there are lines of separation throughout their entire extent (Plate LI, Fig. 3), running in such a manner that single nuclei surrounded by more or less cytoplasm are completely or incompletely mapped out; it is noteworthy that in these cells the lines of cleavage map out more or less distinctly spindle-shaped cells, the long axes of which correspond in direction to the fibres of the stroma. Occasionally the lines separating a nucleated mass are so broad and well marked that it appears as though a distinct cell were lying in a vacuole; the cytoplasm of such giant cells especially seems firm and condensed; the splitting up apparently takes place more slowly, and the impression is received that this may all be caused by the pressure exerted upon the giant cell by the fibrillated stroma in which it is enclosed, or that the cytoplasm is being transformed into the same sort of substance as the fibres of the stroma. The separation of small cells nevertheless continues, because in the older tissue are found small giant cells containing a few—three or four—nuclei and embedded in a dense capsule of nucleated fibrous tissue, without any traceable evidences of nuclear disintegration or of destruction of the giant cells by phagocytes.

When the serum is absorbed rapidly and the giant cells lie in the broad and loose meshes of the new-formed fibrillated tissue, the lines of cleavage may be quite complete, so that single nuclei, surrounded by an irregularly shaped bit of cytoplasm, are split off and imperfect giant cells result, which are surrounded by uninuclear small cells that fit the depressions and elevations on the remnant of the subdividing plasmodial mass. Uninuclear cells thus produced present no signs of necrobiosis and can be traced by means of transition stages into the elongated and flattened cells above described as lying in the new-formed tissue. Nuclear and protoplasmic detritus, as well as evidences of immigration of small cells or leucocytes into giant cells, are not to be observed.

CONCLUSION.

The conclusion seems warranted that the giant cells formed in the absorption of coagulated blood serum inserted into the anterior chamber of the rabbit's eye subdivide again into uninuclear small cells that take part with other new-formed cells derived from the lining of this space to form a densely fibrillated mass of tissue that resembles quite closely the cornea in its structure.

This demonstration materially strengthens the opinion expressed by the writer in his previous article in this Journal,* that the giant cells in healing non-degenerated tuberculous tissue may separate into small living cells, and that the giant cells of tuberculosis are not necrobiotic elements from the very moment and from the very mode of their formation, as has been the general teaching, especially in Germany.

DESCRIPTION OF PLATE LI.

Fig. 1.—Recent giant cell with rounded and smooth margin filled with granules of serum so that the nuclei are covered over.

Photo-micrograph, 500 diameters, of eosin and hæmatoxylin specimen.

Fig. 2.—Photo-micrograph. Separating giant cell lying in the meshes of new fibrous tissue and surrounded by cells and by a cell mass which appear to have been sundered from the larger mass; distinct lines of cleavage are seen in the smaller as well as in the larger mass, so that small uninuclear cells may be said to be separating.

Same magnification and staining as in Fig. 1.

Fig. 3.—Oblong giant cell with a dense protoplasm in which run distinct lines that subdivide the multinucleated mass into more or less well-marked, small, uninuclear cells. In the centre is a part of a cell lying in a distinct vacuole. From a late stage. To the left of the giant cell and the surrounding thick fibres runs the intact posterior elastic lamina, and to the right is the substance of the iris.

Photo-micrograph with same magnification and staining as in Fig. 1.

Fig. 4.—Fusiform thickening of posterior surface of cornea, due to the formation of a densely fibrillated mass of new tissue after complete absorption of the blood serum. The posterior surface of the new-formed mass is clothed with a single layer of endothelial cells which is continuous with the lining of the posterior surface of Descemet's membrane. Close inspection will show the intact posterior elastic lamina running between the new tissue and the cornea.

Photo-micrograph, 100 diameters, of eosin and hæmatoxylin specimen.

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* Loc. cit.

REGENERATION OF NERVE FIBRES IN THE CENTRAL NERVOUS SYSTEM.

By W. L. WORCESTER, M. D., Assistant Physician and Pathologist, Danvers Lunatic Hospital.*

Plate LII.

The tendency of the peripheral nerves to regeneration and restoration of function after division is well known. There would seem to be no reason, *a priori*, why the processes of the neurones within the central nervous system, so similar to the peripheral fibres in structure and function, should not conduct themselves in a like manner; but it is notorious that such is not, at least commonly, the case. Whether the interruption of continuity is due to traumatism or to disease, the rule seems to be that there is no attempt at repair of damage.

In view of this well known fact, it seemed to me that a case coming under my observation, in which there is, in my judgment, beyond reasonable doubt, a growth from interrupted fibres in the central nervous system, deserves to be placed on record. I have been able to find in the literature of the subject but little lending countenance to the belief that such regeneration ever takes place.

Brown-Séquard (1) reported in 1851 a fairly complete restoration of sensibility and partial restoration of movement in a guinea-pig after section of the spinal cord. Microscopical examination of the cord, by himself and Laboulbène, showed very numerous medullated fibres extending through the cicatrix.

Eichhorst and Naunyn (2) in 1874, experimenting on new-born puppies, in which they destroyed the spinal cord by pressure with a blunt instrument without opening the dura, found evidence of restoration of function in some cases, beginning from the third to the fifth week after operation. In from seven to nine weeks the puppies were able to walk on all fours ten or fifteen feet. The movements were very awkward and

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* Read at the meeting of the American Neurological Association, New York, May 28, 1898.

ataxic. These observers thought they got some evidence of restoration of sensibility, but admitted that it was very slight. No further improvement took place; on the contrary, there was generally retrogression after ten or twelve weeks. The histological examination showed in the cicatricial tissue connecting the segments of the cord small numbers of medullated nerve fibres, resembling rather those of the peripheral nerves than of the spinal cord.

Schiefferdecker (3) criticized the foregoing paper severely. Although he did not dispute the presence of newly formed nerve fibres in the cicatrix, he believed that the authors had mistaken reflex for voluntary movements, and denied that there was any restoration of function. His own experiments made on dogs several months old all gave entirely negative results. In consequence of Schiefferdecker's criticisms, Eichhorst (4) took up the subject again. He operated as before on newborn puppies, but isolated the cord and lifted it out of the canal, so that there could be no question of its complete division. He obtained a positive result in only one of three puppies. This one began to move the hind legs at the end of the fourth week; began to walk on the 26th day, and on the 35th day walked continuously a distance of about 180 feet. No evidence of return of sensibility could be found. The animal was killed at that time, and the continuity of the cord was found to be restored by a tissue containing very numerous nerve fibres.

Sgobbo (5) and Marinesco (6) in their experiments by division of the spinal cord both failed to get any evidence, whether functional or anatomical, of regeneration. Popoff (7), in studying the lesions of multiple sclerosis, satisfied himself that the naked axis cylinders found in the lesions were not, as has been generally supposed, remnants of the original constituents of the cord, but, in many instances at least, newly-formed fibres. He claims to have seen the division of a single axis cylinder into a number of fine fibres.

Stroebe (8), experimenting by section of the spinal cord in rabbits, found what he considered unquestionable evidence of growth of nerve fibres into the scar from the posterior nerve roots. In the anterior and lateral columns he found fine medullated fibres in the zone of degeneration, often presenting a contorted, confused course, which he judged to be newly formed. They never passed the line of incision. He concludes that there is an effort at regeneration, but that it never comes to a restoration of continuity in the nervous structure.

Borst and Nicolaier (9), in a case of fracture of the vertebral column in the lower thoracic region in which the patient had survived the injury for three years, found numerous medullated fibres, resembling those of the peripheral nerves, in the region of both the posterior and anterior nerve roots, from which he considers them to be outgrowths, comparing them to the amputation neuromata.

The case to which I ask attention is that of a married woman, aged 41 at the time of her admission to the Danvers Lunatic Hospital, September 28, 1885. Very little information was furnished in regard to her antecedents beyond the fact that she had suffered from repeated attacks of maniacal excitement. At the time of her admission she was much demented; there is no history of any subsequent maniacal outbreak. During her stay in the hospital she had two paretic attacks, both affecting the left side, leaving permanent impairment of motion and sensation, with paræsthesia, which gave rise to delusions that her left side was maltreated in various ways. After the second attack she was confined to her bed until her death, nearly two years afterward.

When she came under my observation in the spring of 1895 there was very marked impairment both of motion and of sensation in all its modes in the entire left side with the exception of the face. She complained of pain in that side, which she attributed to "people throwing things on her." On September 21, 1895, she had an epileptiform convulsion from which she never rallied, dying September 27.

At the autopsy both right corpora quadrigemina were found to be much smaller than normal and of a yellowish brown color. The bodies, together with the pons and medulla oblongata, were hardened in a solution of bichromate of potassium and cut in serial sections for the purpose of studying the secondary degenerations. It then appeared that there was also a large focus of softening in the right side of the pons, involving the pyramidal tract extensively and encroaching on the lemniscus. With the secondary changes from these lesions I am not at present concerned.

On examination of the sections the first-mentioned lesion was found to involve the whole of the right corpora quadrigemina, passing a little beyond the median line above the aqueduct and extending downward laterally to the border of the pes pedunculi. It did not extend below the central gray matter surrounding the aqueduct (see Plate LII, Fig. 1). Microscopically it was seen to consist of a dense felt-work of neuroglia fibres, rather sparsely supplied with vessels.

In the anterior corpus quadrigeminum, approximately in the centre of the diseased area, I found a group of medullated nerve fibres (Plate LII, Fig. 1, *a*) which I at first supposed to be a normal tract which had escaped the destruction that had befallen the surrounding tissue. On tracing it through successive sections, however, I found that the number of fibres diminished in each direction, and that it finally disappeared at each end, without any apparent connection, either proximally or distally, with any normal tissue. On comparing it with the corresponding region on the opposite side, no group of fibres could be found presenting an arrangement at all resembling it. The appearance at the largest portion of the tract can be seen in Fig. 2; near its proximal and distal extremities in Figs. 3 and 4 respectively.

The tract consists of a number of bundles of fibres, showing an irregular, convoluted arrangement, without any apparent order or system. Careful examination of specimens, stained in various ways, failed to reveal any nerve cells in or about this area. In a number of sections, however, small bundles of fibres could be seen extending between it and the zone of white substance surrounding the red nucleus (Plate LII, Fig. 5, *b*). This was the only connection that I could trace between it and normal tissue and must, I think, be the origin of the group in question.

Such are the facts in the case. As to the interpretation to be put upon them, I can see no reasonable hypothesis other than that the fibres in question are outgrowths from neurones that were interrupted in their course by the lesion. That they are not simply a remnant of the original structure seems certain from the fact that nothing at all resembling them can be found in normal specimens from the same region.

Moreover, it will, I think, be evident to any one who studies the specimens that it is entirely improbable that such a tract could be preserved in the very centre of the focus of disease. The appearance of these irregular, contorted bundles of fibres, so strikingly similar to that of an amputation neuroma, is also, to my mind, strongly confirmatory of this view.

If this is the correct interpretation of the facts, it would appear that nerve fibres in the central nervous system, after solution of continuity, may be capable of growth. Of course, in this case, there is no question of regeneration in the sense of restoration of the original structure or function. In the present state of our knowledge this finding must be considered rather as a curiosity than a matter of practical importance.

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DESCRIPTION OF PLATE LII.

Fig. 1.—Section through corpora quadrigemina, × 2. Newly formed fibres at *a*.

Fig. 2.—Fibres at *a* in preceding figure, × 40.

Fig. 3.—Newly formed fibres near proximal end, × 40.

Fig. 4.—Same, near distal end, × 40.

Fig. 5.—Same, thickest portion of tract. Bundle of fibres connecting it with tegmentum at *b*.

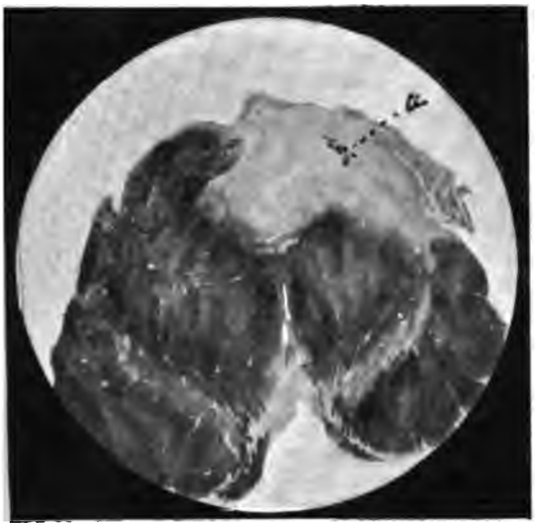

FIG. 1.

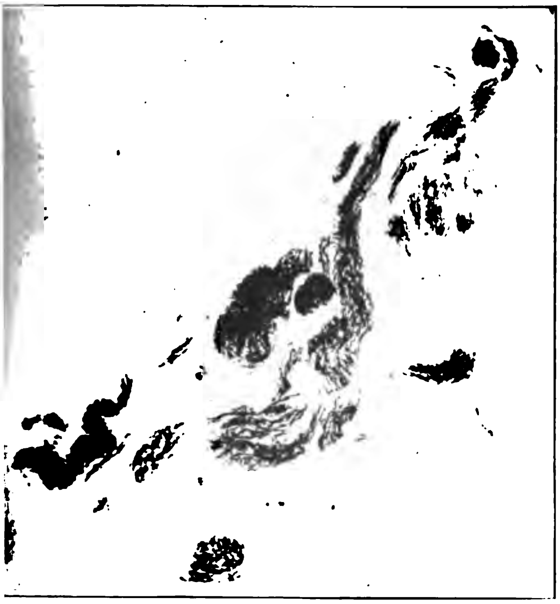

FIG. 2.

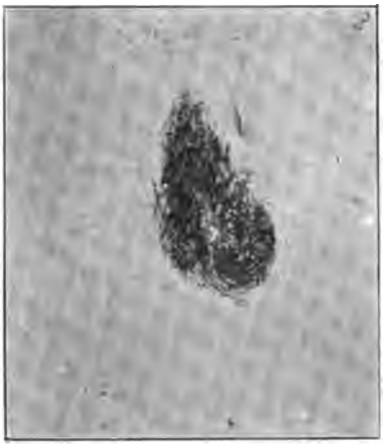

FIG. 3.

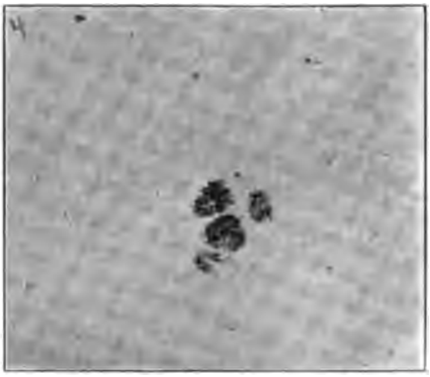

FIG. 4.

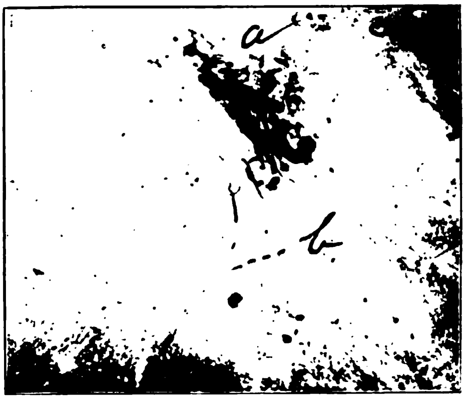

FIG. 5.

EXPERIMENTAL FAT NECROSIS: THE ACTION OF PANCREATIC TISSUE UPON SUBCUTANEOUS ADIPOSE TISSUE.

By HERBERT U. WILLIAMS, M. D.

(*From the Pathological Laboratory of the University of Buffalo.*)

PLATE LIII.

In a paper by the writer published in 1897* an account was given of some experiments which sought to discover the cause of the "fat necrosis" so often associated with acute disease of the pancreas. Since fat necrosis consists of a decomposition of the neutral oils contained in fat cells, the attempt was made to discover whether that change could be accomplished by the pancreatic juice, which has a ferment capable of decomposing neutral oils. To this end a ligature was placed about the gastro-splenic portion of the pancreas near the duodenum. In some cases as many as possible of the veins leaving the pancreas were tied and the substance of the gland was lacerated with a sharp hook. The object of the operation was to prevent the outflow of the pancreatic juice into the duodenum and to permit it to act upon the adjacent adipose tissues. The operation was performed upon twenty animals, mostly cats. In five cases well-marked fat necrosis ensued. There were three indecisive and twelve negative results. In three of the five cases where positive findings were obtained an accidental micrococcus infection was associated. It was not, therefore, considered justifiable to attribute the fat necrosis to the action of the pancreatic juice alone.

In order to discover whether bacteria played any important role in the production of fat necrosis, operations similar to those described have since been undertaken, where infection with pyogenic bacteria was brought about intentionally.

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* *Bost. Med. and Surg. Journ.*, April 15, 1897.

Cat 37. Large, fat. The gastro-splenic portion of the pancreas and some of the veins issuing from it were ligated, the pancreas was lacerated with a sharp hook, and its surface was smeared with a quantity of the staphylococcus pyogenes aureus from a fresh culture on agar. Upon the following morning the cat was found dead. The autopsy showed beginning peritonitis and widely spread fat necrosis in the adipose tissue near the pancreas and in the omentum, in the form of a thin, flat layer, including the most superficial cells just below the peritoneum. The staphylococcus pyogenes aureus was recovered in cultures from the peritoneum, liver and spleen. Examination of the other viscera yielded nothing noteworthy. Microscopic sections showed well-marked beginning pancreatitis.

Cat 44. Medium sized, fairly well nourished. The gastro-splenic portion of the pancreas was ligated close to the duodenum, also several veins leaving it; it was lacerated with a sharp hook, and the surface was smeared with the streptococcus pyogenes from a culture made recently from pus on agar. On the fourth day the animal was found dead. Autopsy revealed peritonitis, especially near the pancreas. A part of the tail of the pancreas was disorganized and necrotic. There were numerous evidences of fat necrosis in the retro-peritoneal fat of the pancreatic region and in the omentum, and a few scattered spots appeared in the mesentery and meso-rectum. Some of the areas were large and flat, others were localized. The lungs showed pneumonia and fibrinous pleurisy. Smears from the peritoneum yielded a streptococcus and a large bacillus. The streptococcus alone was recovered in cultures. The evidences of acute pancreatitis were very marked in microscopic sections.

Cat 45. Large, well nourished. The gastro-splenic part of the pancreas was ligated; there were no laceration and no ligation of the veins, but the surface was smeared with the staphylococcus pyogenes aureus. The animal remaining healthy in appearance was killed at the end of a week. A single small area of fat necrosis was found close to the pancreas. Localized opacities due to thickening of the peritoneum were seen, but there was no general peritonitis. Pancreatitis was well marked.

The proportion of positive results was larger from these than from the first series of operations, but the number of cases was too small to permit one to draw far-reaching conclusions. The extensive fat necrosis in Cats 37 and 44 seemed as likely to have been due to the laceration of the pancreas as to the introduction of the pyogenic cocci, though the latter may have served to hasten or intensify the result.

It will be noticed that in Cat 37 extensive fat necrosis occurred within twenty-four hours after the operation on the pancreas. As following this line of procedure did not seem likely to lead to a solution of the problem under consideration, it was abandoned. Furthermore, on account of the instability of the fat-splitting ferment, injections of pancreatic extracts appeared to offer an unpromising prospect.

In an experiment described in the article already mentioned a large piece of fresh cat's pancreas was inserted into the abdominal cavity of another cat, with the view of observing the effects of its ferments on the peritoneal adipose tissues. Although fat necrosis followed, an accidental infection of the peritoneum and of the animal's own pancreas also took place and nullified the results. Owing to the danger of thus confusing the possible effects of disease of the animal's own pancreas with those of the pancreas artificially introduced, the peritoneal adipose tissue was deemed an unsuitable location, and efforts were then made to insert pieces of pancreas into the subcutaneous or intermuscular fat. As before, the cat was the animal selected. Numerous attempts to cultivate bacteria from the fresh cat's pancreas planted upon agar and kept in the incubator found it to be sterile in each case. In most of the following experiments the pancreas used was tested for its fat-splitting power, always with positive results. The locations chosen for the operations were the inguinal regions and a point a little below the sternum. The skin of the vicinity was shaved and washed with alcohol and an antiseptic solution. The instruments and silk used were sterilized with heat. The pancreas was taken fresh and with great care from the abdomen of another cat. It was inserted into the adipose tissues through a small incision, and was held in position by a silk suture, not passing through it, but uniting the edges of the tissues at the bottom of the wound. The superficial incision was closed separately with silk and collodion.

Cat 27. Thin. A piece of fresh pancreas from another cat was introduced through a small incision into the subcutaneous adipose tissue of the right inguinal region. After four days a large abscess had resulted, undoubtedly from accidental infection. The animal was killed and the abscess removed entire for histological examination. Besides the ordinary changes of suppuration, the walls of the abscess showed areas of fat cells presenting a reddish blue stain with hæmatoxylin and eosin and contents having the homogeneous appearance seen in undoubted fat necrosis. Some cells exhibited large needle-shaped crystals. Owing to the occurrence of suppuration the result was considered indecisive, but as probably indicating fat necrosis.

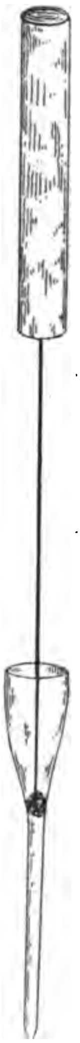
Cat 30. Healthy, young animal, quite fat. Portions of fresh pancreas from another cat were introduced into the subcutaneous adipose tissue of the right inguinal region and into the adipose tissue below the sternum behind the linea alba. After four days the points of introduction had become immense abscesses. The animal was killed and both abscesses were removed entire. The histological examination gave the same result as in the last case.

Cat 33. Healthy young cat, well nourished. Three localities were utilized for the introduction of cat's pancreas into the adipose tissues: the middle line below the sternum, the middle line above the symphysis pubis, and the right inguinal region. After five days abscess formation had begun, so that the animal was killed and the abscesses were excised. The histological examination detected no fat necrosis.

Cat 35. Large, well nourished. A piece of fresh cat's pancreas was introduced below the sternum in the same manner and with the same precautions as previously, except that a portion of adipose tissue was excised to make room for the piece of pancreas. After three days an immense abscess had resulted. The animal was killed and the abscess removed. The examination of sections with the microscope gave the changes to be expected for suppuration, with alterations in the fat cells similar to those already mentioned for cats 27 and 30.

The experiments just described seemed to show the futility of continuing along the lines followed in them. Although cultures were not made from the abscesses encountered, it was sufficiently evident that they proceeded from infection. In cases related hereafter, where identical results were obtained, the micro-organisms causing the suppuration were demonstrated. (Cats 43 and 50.) It was impossible to determine how far the changes in the tissues were to be attributed to bacteria, and how far to the working of the pancreatic ferments. As has been remarked, the infection probably took place from organisms on the skin, as the pancreas was removed with such care as to render it above suspicion. The writer has been led to suspect that the pancreas may be possessed of irritating properties which make the tissues in contact with it extremely liable to infection.

In order to observe the effect of perfectly sterile pancreas on the tissues the following technique was devised, which can be recommended when it is desired to insert solid substances hypodermically. A cannula of glass of the size and shape represented in the accompanying figure was made from ordinary glass tubing. It was sterilized in a Petri dish, and the portion of pancreas to be used was placed in the large end, with a bit of sterile black silk. The pancreas and silk were forced along to the small end with a stiff platinum wire. The skin having been shaved, cleaned and rendered aseptic as far as possible, a small incision was made through it. The adipose tissues underlying it were nicked. The small end of the cannula was then forced into the adipose tissue three or four centimetres. The pancreas and bit of silk were pushed out of it by the wire, depositing them in the desired locality, after the withdrawal of the cannula. The surface wound was closed with one or two sutures. The success of this plan was very gratifying. In seventeen experiments made according to it upon nine cats, eleven were satisfactory in excluding bacteria, while infection occurred in but four instances and two experiments in another animal indicated doubtful infection. The black silk served admirably to identify the point of introduction.



Cat 38. Large, fat. Pieces of fresh pancreas one to two millimetres in diameter were inserted into the adipose tissues just below the sternum and in the right inguinal region, after the manner just described. On the twelfth day there had been no evidence of inflammation, and the wounds in the skin were nearly closed. The animal was killed. The points where the pancreas had been inserted were at once located by means of the black silk. Agar tubes were inoculated from both, and placed in the incubator. These tubes remained sterile. Serial sections of both regions were cut; those from below the sternum were negative; in the inguinal region a small area of fat necrosis was found.

Cat 39. Large, fat, not very vigorous. Pieces of fresh cat's pancreas one to two millimetres in diameter were inserted through the sterile cannula into the adipose tissues of the right groin and below the sternum. The wounds of the skin healed rapidly and without incident. The animal was killed three weeks after the operation. Cultures were made from both points, which remained sterile. The examination of sections showed no fat necrosis.

Cat 40. Small, thin. A small piece of fresh cat's pancreas was placed in the fat tissue of the right groin through the cannula. The wound remained healthy. The cat was killed at the end of six days. Cultures from the locality remained sterile. Examination of sections did not demonstrate fat necrosis.

Cat 42. Small, not fat. By means of the sterile cannula small bits of fresh cat's pancreas were introduced into the adipose tissue in the sternal and left inguinal regions. After the lapse of seventeen days the points in question were examined. A positive result was obtained in the left groin, where a small spot of fat necrosis was found. Agar tubes from this area showed no growth. No bacteria could be found in sections stained by the Gram-Weigert method and with methylene-blue.

Cat 43. Large, fat. Through a glass cannula, bits of fresh cat's pancreas were inserted in the adipose tissues in three places: the right and left inguinal regions and the region below the sternum. At the end of three days there was much swelling and slight fluctuation. The cat was killed. Smears were made and agar tubes inoculated from all three localities, and the spots were excised. Suppuration was evident in all, and gas formation, as well, below the sternum. The pancreas taken in this case was from the same source as that used for Cat 42 and had produced no growth on agar. As Cat 42 had shown no suppuration, the source of infection in Cat 43 was probably the skin. The cause of the suppuration proved to be an undetermined micrococcus. Examination of sections showed probable fat necrosis in both inguinal regions, but none in the locality below the sternum.

Cat 47. Small, sickly, not fat. Pieces of fresh pancreas of a young cat were placed in the right and left inguinal adipose tissues through the glass cannula. The cat died unexpectedly two nights later. Autopsy showed the cause of death to be bronchitis and broncho-pneumonia, probably having no relation with the operation. The edges of the wounds had parted slightly, but there was no swelling in the neighborhood, nor any of the macroscopic evidences of suppuration. Cultures from both sides yielded a small bacillus, resembling B. coli communis. The weather during this period was excessively hot, and the results of the cultures were not deemed positive evidence of ante-mortem infection of the points of introduction. On both sides, in the neighborhood of the inserted bits of pancreas and for two to three millimetres in the track taken by the cannula, the adipose tissue had a strikingly white, opaque, cheesy appearance. Examination with the microscope, fresh and in sections, demonstrated the histology of fat necrosis.

Cat 48. Large, moderately fat. The fresh pancreas of a half-grown kitten was used. Small bits were inserted into the adipose tissues of the right and left inguinal regions through the glass cannula. At the end of five days the cat, appearing healthy, was killed. The regions where the pancreas had been placed were not swollen; but the adipose tissues for two or three millimetres around presented a white, cheesy appearance. Examination of the cheesy matter fresh, and of the region in sections, showed the characters of fat necrosis. Smears and sections stained for bacteria and culture tests all gave negative results.

Cat 49. Medium sized. Bits of fresh pancreas from a full-grown cat were introduced by means of a sterile glass cannula into the adipose tissues of both inguinal regions. After eight days, appearing healthy, the cat was killed. The skin wounds were in good condition. There was slight swelling where the pancreas had been placed, but no suppuration. There were, however, numerous minute, curd-like, white spots on the edges of the adjacent adipose tissues. Agar tubes inoculated from the region remained sterile. Smears stained for bacteria gave negative results, as did the subsequent examination of sections of the tissue for bacteria. Inspection of the curd-like masses and study of sections of the tissues gave the histology of fat necrosis.

Cat 50. Small. Received a bit of fresh cat's pancreas in the inguinal region through the sterile glass cannula. At the end of eight days swelling and fluctuation indicated pus formation. The pus contained an undetermined diplococcus. Nevertheless, the histological characteristics of fat necrosis were demonstrated in fresh preparations and in hardened and stained sections.

Summary.—Thirteen cats were used for the subcutaneous introduction of pancreas into the adipose tissues in twenty-four places. At nine points fat necrosis ensued; in nine there was no fat necrosis; in six the result was doubtful. The doubtful results were in cases in which suppuration complicated the issue. At eleven points there was no infection, two were doubtful, and eleven became infected. Selecting the eleven in which there was no infection and the two classed as doubtful, where infection was probably post-mortem, in all thirteen, we find eight points at which fat necrosis occurred and five from which it was absent.

General description of the lesions at the site of introduction of the pancreas.—In those cases which resulted favorably and where the autopsies were performed only a few days after the subcutaneous insertion of the pancreas, the adipose tissues in that vicinity, which was identified by the piece of black silk, acquired a white, opaque and cheesy character.

The opacity was found to reside in certain fat cells; while adjacent fat cells presented a normal appearance. The altered cells exhibited a brown tinge, and their contents consisted of minute linear crystals. Ring-shaped plates were seldom seen, while they were common in the necrosis obtained in the cat after operations on the pancreas. Fragments of similar opaque material were seen which appeared to have arisen from the breaking up of such altered cells. Loose crystals and granules were also abundant.

The amount of material was too small to permit the application of micro-chemical tests in a perfectly satisfactory manner in all instances. When they could be employed the reactions were those characteristic for fat necrosis, including sometimes the demonstration of calcium salts.

The tissues were hardened in alcohol or in formaldehyde and embedded in celloidin. Sections stained with hæmatoxylin and eosin gave satisfactory pictures. The region where the pancreas had been placed was readily discovered by means of the black silk which had been introduced with the piece of pancreas. The pancreas itself could not be identified with certainty. Fragments of disorganized tissue associated with the bits of silk, staining with eosin, not stained by the Gram-Weigert method, and exhibiting neither nuclei nor definite structure, were supposed to represent the pieces of pancreas.

The altered fat cells were found on the surface of the cavity containing the silk and these fragments of tissue (Plate LIII). Their contents stained intensely, partly with hæmatoxylin, partly with eosin. The contents consisted in some cases of homogeneous material, in some cases of needle-shaped or fine feathery crystals, plainly visible in balsam preparations. They often contained granules, which stained intensely with hæmatoxylin, of very variable size, usually round, but sometimes quite irregular in form. Similar granules occurred between the cells. Some of these granules may have owed their intense stain to the presence of calcium salts. Some of them appeared to have arisen from the breaking up of nuclei. Nuclei belonging to the necrotic fat cells could not be discovered. The altered fat cells were of normal size or slightly enlarged at most. Necrotic cells were found, which were almost completely disintegrated, and with them loose crystals and fine granular material.

Between and around the necrotic fat cells polynuclear leucocytes appeared, usually so as to make an extensive accumulation, though sometimes in relatively small numbers. The interstices of the neighboring unaltered adipose tissues also contained leucocytes, which, with the granules above mentioned, occasionally constituted a well-marked zone around the region of operation. Larger cells with undivided nuclei, probably endothelial, were visible in smaller numbers, especially around the fibres of silk. Fragmentation of nuclei was often seen. A fine reticulum, stained by the Gram-Weigert method, which was observed sometimes in the area of most intense necrosis, suggested the formation of fibrin. In no case was there marked evidence of hæmorrhages. Where the time after operation was longest (12 days in Cat 38, 17 days in Cat 42) the silk and area of necrotic cells were enclosed in richly cellular, new-formed fibrous tissue. In the same cases there was less accumulation of leucocytes than in the more recent ones.

It will be seen that in all essential respects the histological characters of subcutaneous adipose tissues which are subjected to the direct action of the excised pancreas may be identical with the peritoneal fat necroses observed after ligation, laceration, and other operations on the pancreas. The probability, therefore, becomes very strong that fat necrosis may be produced by the unaided action of the ferments of the pancreas.

DESCRIPTION OF PLATE LIII.

Fig. 1.—Cat 47. Left inguinal adipose tissue, showing unaltered and necrotic fat cells, accumulation of granules and leucocytes at point of junction, black silk and dark fragment of tissue close to it, probably pancreas; two days after insertion of pancreas. Hæmatoxylin and eosin. Photomicrograph by H. M. Hill. Spencer ⅔-inch objective. ×70.

Fig. 2. Cat 42. Part of the area of fat necrosis from the left inguinal region; seventeen days after insertion of pancreas. Photo-micrograph by H. M. Hill. Spencer ⅔-inch objective. ×70.

Fig. 3.—Cat 48. Left inguinal region, showing isolated fragment of adipose tissue, almost wholly necrotic, and leucocytes. Hæmatoxylin and eosin. Low-power water-color sketch by Miss E. M. Porter.

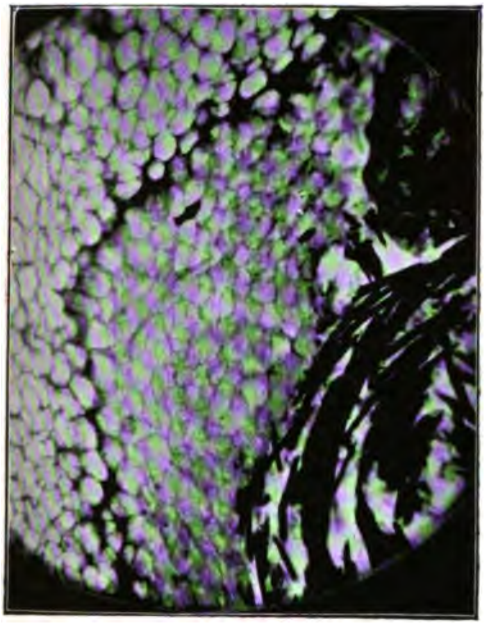


FIG. 1.

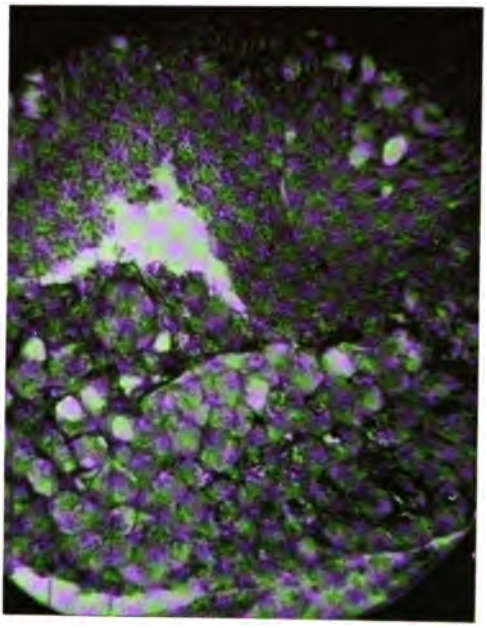


FIG. 2.

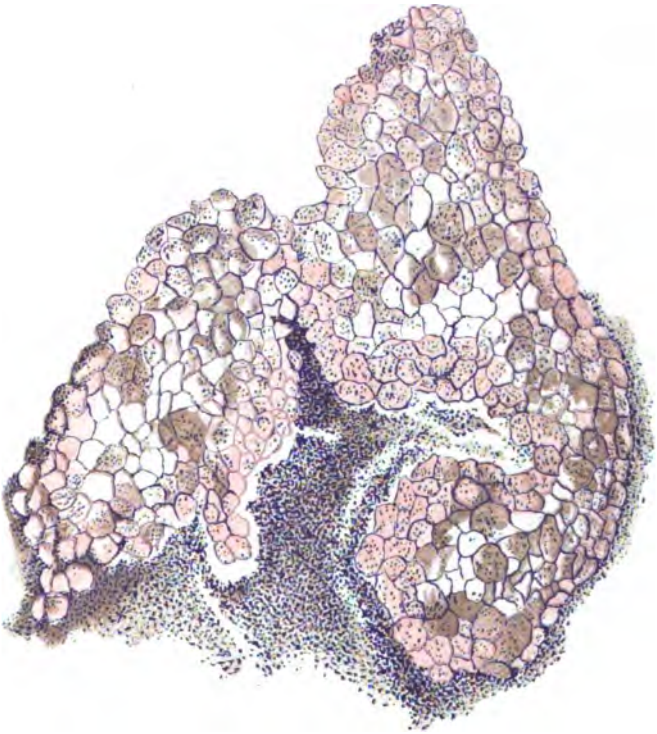


FIG. 3.

A CONTRIBUTION TO THE STUDY OF THE PATHOGENESIS OF THE BACILLUS PYOCYANEUS, WITH SPECIAL REFERENCE TO ITS RELATION TO AN EPIDEMIC OF DYSENTERY.

By AUGUST JEROME LARTIGAU, M. D.

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The bacillus pyocyaneus has within recent years attracted attention owing to the results of experimentation with this organism, and also to the publication of a number of isolated instances demonstrating its relation to certain infectious processes in man.

The occasional blue or green staining of surgical dressings has been known for a long time, and the nature of the coloring principle was early ascertained by Fordos,* who was successful in isolating it from such dressings. The cause of the production of the pigment was not, however, discovered until much later, when Gessard,† after a number of unsuccessful attempts by several other investigators, was able to demonstrate that the coloring matter was manufactured by the bacillus pyocyaneus, which he succeeded in growing in pure culture for the first time. Subsequently to Gessard's discovery this bacillus was for some time regarded as a purely saprophytic micro-organism incapable of producing pathological lesions either in animals or in man. Ledderhose‡ was among the first to study experimental infections with the bacillus pyocyaneus, but the present conception of its etiological relations to the production of certain definite pathological processes is due to Charrin's§ admirable researches.

Charrin in his experimental studies of the organism upon animals was able to observe with considerable constancy a marked disposition toward a definite clinical evolution as manifested by the appearance of symptoms which resulted in the presentation of a fairly characteristic clinical entity. To this symptom-complex he gave the name "maladie pyocyanique." In rabbits, even when subcutaneous inoculation is resorted to, the systemic symptoms predominate, and it is in this animal that the disease is presented in its most typical form. The symptoms generally noted are diarrhœa, albuminuria, fever, and in the more chronic cases, cachexia and paralyses, which Charrin considers of a perfectly characteristic type, and in which the hind quarters are the ones most frequently involved. The paralysis is essentially spastic in nature. At autopsy the animals show congestion of the mucous membrane of the intestine and usually small hæmorrhages in the various viscera, but more constantly in the intestinal walls. Schäfer,[*] although he carried on his experimentation with the dog, arrived at results not unlike those obtained by Charrin. Cadéac,[†] shortly after the publication of Charrin's results, reported a case of spontaneous pyocyaneus infection in the dog. Since this there has been an abundance of experimental evidence which is essentially confirmatory of the conclusions of Charrin, Ledderhose and Schäfer.

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* *Compt. rend. Acad. des Sciences*, 1860, li.

† La pyocyanine. Thèse de Paris, 1882.

‡ *Deutsch. Zeitschr. f. Chirurgie*, xxviii, 1888.

§ La maladie pyocyanique. Paris, 1889.

The bacillus pyocyaneus has been found in the saliva and in sputum by Pansini[‡] and Frick,[§] who attribute the green color sometimes observed in sputum largely to this bacterium. It has also been detected in the sweat by Eberth[¶] and Audouard.[||] Abelous[**] found it in the stomach, and its isolation from wounds from time to time has been already mentioned. Its presence in suppurating wounds is rather infrequent, as shown by Jakowski,[††] who was able to find it in his bacteriological examinations only twice in 200 cases of this nature. This same observer obtained it in almost pure culture from a fistula of the large intestine, and a second time from a fistula of the small intestine. Observations of Koch showed the presence of the bacillus in tuberculous cavities.

Investigators at this time generally believed that its presence and association with pathological conditions were only as a saprophyte. The conception of the bacillus pyocyaneus as an etiological factor, which possibly might at times excite morbid phenomena, was reserved for

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[*] Inaug.-Diss. Berlin, 1891.

[†] *Comptes rend. de la Soc. de biol.*, 9. s., ii (1890), 41.

[‡] Virchow's *Archiv*, cxxii, 424.

[§] Ibid., cxvi (1889), 266.

[¶] *Medicin. Centralbl.*, 1873, and Virchow's *Archiv*, lxii (1875), 512.

[||] *Journal de médecine de l'Ouest*, 1879.

[**] Thèse de Montpellier. 1888.

[††] *Zeitschr. f. Hygiene*, xv, 475.

Gruber,* who obtained the organism from a case of middle-ear disease. The recognition of its power to produce pathological conditions has more recently received elucidation in the observations of a number of clinicians and experimenters.

Martha† reports two cases of purulent otitis media from which pure cultures of the bacillus were obtained. It has likewise been cultivated from many other cases of otorrhœa, especially in children, sometimes in pure culture, at other times in association with other organisms, by Maggiora and Gradenigo,‡ Babes,§ Pes and Gradenigo,¶ Finkelstein,|| and others. Kossel,** in the bacteriological examination of 52 cases of purulent middle-ear disease, isolated it eight times, and in three of these cases was able to cultivate it from the blood. Charrin†† found it in a case of puerperal fever associated with a few streptococci and also isolated it from a case of chronic mastitis. Blumer‡‡ cultivated the organism in practically pure culture from a case clinically resembling diphtheria; Monnier§§ from the saliva in noma and also from a meningeal exudate. Babes¶¶ reported a localized pyocyaneus infection of the umbilical cord, while H. C. Ernst|||| obtained the bacillus from the pericardial exudate during life from a case of tuberculous pericarditis. In addition to these cases of local infection, it has been demonstrated in an ovarian abscess by Barker,*** and the same writer has studied it in diphtheritic inflammation of the œsophagus, broncho-pneumonia, ureteritis, pyelonephritis, and intestinal ulceration, as well as in cases of general infection. In his paper Barker also cites two cases of hepatic abscess reported by Kruse and Pasquale. The micro-organism has been isolated from a number of cases of urinary infection by Jadkewitsch,††† Oettin-

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* *Monatschr. f. Ohrenheilk.*, No. 7, 887.

† *Arch. de méd. expér.*, iv (1892), 130.

‡ *Ann. de l'Instit. Pasteur*, t. v.

§ Cornil et Babes. Les Bactéries, p. 486.

¶ *Gazz. med. di Torino.*, xlv (1894), 521.

|| *Charité-Annalen*, p. 346, 1896.

** *Zeitschr. f. Hygiene*, xvi, 1894.

†† Loc. cit.

‡‡ *Johns Hopkins Hosp. Bull.*, 1895.

§§ *Gaz. méd.*, Nantes, 1895.

¶¶ Loc. cit.

|||| *Am. Jour. Med. Sci.*, cvi (1893), 396.

*** *Jour. Amer. Med. Assn.*, 1897.

††† Baumgarten's *Jahresbericht*, p. 355, 1890.

ger,[*] B. Motz,[†] and Le Noir,[‡] who obtained it either in pure cultures or associated with other bacteria.

It is evident from the data at hand that the bacillus pyocyaneus is capable of causing a whole series of localized lesions, acute or chronic, of very different sorts, being in this respect not unlike the common pus cocci. The causative relation of this organism to such a large number of processes whose diversity is so considerable, as in the case of the pus cocci, is largely dependent upon the multiplicity of its sources of entrance into the body.

The ability of the organism to produce a general febrile process which may terminate fatally has been well demonstrated by the clinical and bacteriological observations of the past few years. Ehlers[§] was the first to draw attention to this by the publication of an instance of general systemic infection. His views received confirmation from Neumann[¶] and Oettinger,[||] both of whom reported cases of this nature. Later Karlinsky[**] published an interesting observation of generalized pyocyaneus infection in which the bacillus was obtained in pure culture from the skin vesicles, spleen, blood and Peyer's patches. Monnier[††] studied an interesting example of this variety of infection in a case of broncho-pneumonia with pleurisy, in which he isolated the bacillus of blue pus from the heart's blood, pleural exudate and from the consolidated areas of broncho-pneumonia. Kossel,[‡‡] Krannhals,[§§] Calmette,[¶¶] Williams,[||||] Finkelstein,[***] Barker[†††] and the author[‡‡‡] have studied other illustrative cases of general pyocyanic infection in children and in adults.

Recently Triboulet[§§§] observed an instructive case belonging to this class in a child in which the systemic invasion was secondary to an impetigo and ulcerative ecthyma, from both of which lesions the pyocyaneus was isolated during life. The writer obtained from the laparotomy wound in a case of acute peritonitis the organism in pure culture and from the purulent peritoneal contents associated with a few colon bacilli. Cultures from the blood contained it in pure growth. From a case of acute pleuritis we also obtained it in pure culture. Jadkewitsch's case* is a most curious example of the nervous type of infection with this micro-organism.

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[*] *Semaine Méd.*, p. 385, 1890.

[†] *Compt. rend. Soc. de biol.*, p. 128, No. 4, 1896.

[‡] Ibid., 1896.

[§] *Hosp. Tid.*, Kjöbenh., 1890.

[¶] *Arch. f. Kinderheilk.*, Hft. 1 and 2, 1890, xii, 1891.

[||] Loc. cit.

[**] *Prager med. Woch.*, p. 231, No. 20, 1891.

[††] See Legars. Essai sur la maladie pyocyanique chez l'homme. Thèse de Paris, 1895.

[‡‡] Loc. cit.

[§§] *Deutsche Zeitschr. f. Chirurg.*, xxxvii, 181, 1893.

[¶¶] *Arch. de méd. navale et coloniale*, April, 1892.

[||||] *Montreal Medical Journal*, July, 1894.

[***] Loc. cit.

[†††] Loc. cit.

[‡‡‡] *Philadelphia Med. Journal*, Sept. 17, 1898.

[§§§] *Comptes rend. Soc. de biol.*, Oct. 22, 1897.

Many of these instances of generalized invasion presented symptoms not unlike those observed in the experimental forms of infection; indeed, the similitude in some of the cases is most striking. A pronounced hæmorrhagic diathesis was noted in the majority of these cases, manifesting itself either in the form of hæmorrhages into the skin or sometimes into the internal organs, particularly into the intestinal wall. Diarrhœa was not uncommon and congestion of the intestinal mucosa of considerable intensity was noted in a few of the cases.

The infrequency of infection with this micro-organism is well shown by the fact that Krannhals† in the investigation of 30 cases of general septic and pyæmic infections was not able in a single instance to demonstrate the presence of the bacillus pyocyaneus. In the systematic bacteriological examination in 800 consecutive autopsies at the Johns Hopkins Hospital, Barker‡ found but 11 cases in which there was infection with this organism, sometimes manifesting itself as an acute local, at other times as a generalized process.

The objections advanced against the pathogenesis of the bacillus pyocyaneus in man by Schimmelbusch§ in his critical article certainly can no longer be regarded as tenable with the important affirmative evidence in our possession at the present day—all clearly demonstrating a more or less marked capability of the bacillus to produce lesions in the human subject.

The epidemic of dysentery, the report of which is the chief purpose of this paper, was investigated for the New York State Board of Health. For the clinical features of the cases we are indebted to Dr. M. C. Wright, of Mt. Vision, N. Y.

General Features.—The epidemic occurred at Hartwick, a small hamlet in Otsego county, New York. It was limited to 15 individuals, with 11 recoveries and 4 deaths. 14 of these were members of two separate families living about one mile from each other. Little or no communication existed between the two houses. The first patient was attacked August 23, 1897, and the epidemic continued during the remainder of that month and the whole of September.

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* Loc. cit.

† Loc. cit.

‡ Loc. cit.

§ Volkmann's *Samml. klin. Vorträge*, N. F., No. 62, 1893.

The disease first appeared in the S. family, composed of seven members, who all suffered from the affection, to which three succumbed, a male aged 35 and two children aged respectively 19 months and 5 years. The others made a good recovery after a long and tedious convalescence. The disease appeared in the second family about three weeks after the first member of the S. family was taken sick, and likewise attacked seven individuals, of whom six recovered and one, a child four years of age, died on the thirteenth day of the disease. The fifteenth case, it is interesting to note, was the physician in attendance on the S. family. On his first visit he drank freely of water, and three days subsequently was taken ill with symptoms in every manner identical with those presented by his patients.

Clinical Features.—Following is a short history of the clinical features of each case as given me by Dr. Wright:

Case 1. Duam S., age 35 years. Taken sick August 23 with pain in bowels and diarrhœal discharges which soon became bloody. Great tenderness over colon and marked tenesmus. Passages very frequent, one about every hour until death. Temperature ranged between 99° and 102° F. Death September 10.

Case 2. Milo S., age 19 months. Taken sick on same day as the first patient. Frequent bloody passages and great tenesmus. Much abdominal tenderness and considerable vomiting. Temperature 100°-103° F. Death August 26.

Case 3. A. S., 2 years. Illness commenced August 27. Symptoms milder than in either of the two preceding cases. Temperature did not go above 101° F. The duration of the disease was about three weeks and was followed by complete recovery.

Case 4. Ella S., age 37 years. Disease began September 1 and continued for about two weeks. Tenesmus and pain along line of the colon. Temperature at no time higher than 101° F. Recovery.

Case 5. Alice S., 5 years old. Taken sick September 7 and died on the third day of her illness. Tenesmus marked and passages very bloody and frequent.

Case 6. Mrs. Duam S., 30 years. Taken sick September 10 with frequent passages (about 15 to 20 per day); tenesmus; temperature not over 100° F. Tenderness over large bowel was very acute, especially over cæcum. Vomiting quite frequent during first week. Recovery after 12 days' illness.

Case 7. Mrs. Van B., 50 years. Disease commenced September 14 and lasted about three weeks. Symptoms similar to those observed in Case 6, vomiting being perhaps still more severe. Practically no fever. Recovery after a very long convalescence.

Case 8. Mr. G. A., 20 years of age. Taken sick September 14 with diarrhœa, which quickly became bloody. Temperature on 15th reached 104° F., with a pulse rate of 120 to 130. For the first three days the condition was very critical, but after this improvement was slow but progressive, so that by the fourth week he was practically well.

Case 9. Mrs. W., about 28 years. In this case the symptoms were essentially those observed in the preceding cases, except that the clinical manifestations were less intense. Recovered in about two and one-half weeks.

Case 10. Mrs. W., 34 years. This patient had been nursing the S. family for a few days during their illness and shortly after returning home was in turn taken sick. The symptoms were severe for two days, but a marked amelioration followed shortly thereafter, so that by the end of the second week she had recovered.

Case 11. Florence C., aged 8. Sickness commenced September 17, and the symptoms continued for seven days. Attack very mild. Diarrhœa but no blood in stools. Recovery.

Case 12. Mr. C., 40 years of age. The sickness was ushered in with a chill. Diarrhœa for two days. Stools then became bloody. Marked tenesmus was present in this case. Frequent vomiting during first week. The disease lasted about six weeks, which was longer than in any of the other cases. Convalescence has been exceedingly slow.

Case 13. Maud C., 4 years. Illness began on the 24th of September and continued for twelve days. Frequent passages containing more or less blood, reaching often as many as 20 in a day. Vomiting and tenesmus very marked. Temperature not over 101° F. Hæmorrhage from the gums occurred in this case on the tenth day. Death on October 6th.

Case 14. Amos C., about 9 years old. Taken sick on September 26. Clinical picture practically same as in the other mild cases, except that patient passed more blood. Recovered.

Case 15. Dr. W., the physician to the S. family, was taken ill about September 1 with symptoms of a mild character, but of the same nature as those observed in the majority of the cases. Symptoms persisted for about nine days, when recovery took place.

It is seen, then, that all of the cases presented certain symptoms in common, so that the clinical pictures in the several instances bear a striking resemblance to each other. The onset was acute and the disease was ushered in sometimes with a chill, otherwise always with diarrhœal symptoms, which soon changed to those of a dysenteric type, characterized by frequent watery passages containing a greater or less amount of blood. Tenesmus and tenderness over colon were quite marked in some cases. Temperature was high in one instance (Case 8, 104° F.), but in the others it was never higher than 102° F. The duration of the disease in the different cases was quite variable, but the average was about three weeks for the non-fatal cases. One patient died on the third day of her illness. Vomiting, although not present in all cases, was, nevertheless, a marked symptom in many of the cases, particularly those presenting the more severe forms of the affection. An interesting and suggestive point which was noted about the physical appearance of the stools, but not mentioned in the histories, was the frequently distinct green color of the passages. This color became most evident after exposure to the air, and only rarely was it noticed at time of passage, and in these cases it was but faintly marked. Calmette in his studies had noticed this and explained it as due to oxidation of the pyocyanine contained in the intestinal dejecta. No autopsies could be obtained upon any of the fatal cases.

Hygienic Surroundings.—The two habitations are situated in each instance very favorably with relation to the formation of the surface of the soil, but the immediate surroundings and habits of each family are in every manner suggestive of an extremely low degree of hygienic morale, and close inspection and examination confirmed this in every way. In each family the well for the supply of drinking water is situated in close proximity to the kitchen door and is more or less imperfectly covered over with timber and earth. The soil over the well and immediately adjoining ground was covered by a layer of decomposing vegetable and animal detritus of offensive odor.

These substances were derived mostly from the kitchen, as it is the habit in each family to rid themselves of the refuse and other slops by throwing them, usually from the kitchen door, upon the ground in the vicinity of the supply of their drinking water. It is easy to understand that contamination of this well-water was a matter of no great difficulty, since the soil covering the well is only about two feet in depth and mostly made up of coarse sand through which penetration is not difficult. For none of the wells used for the supply of drinking water at the time of the epidemic could any other possible source of contamination be made out. At the C. house there exist two other wells located in a more favorable situation, but nevertheless readily liable to outside contamination by reason of their open character. Neither of these wells were used for the supply of drinking water at the time of the epidemic or previous to its occurrence.

The investigation was necessarily restricted in its extent owing to the distance of, and difficult accessibility to, the seat of the epidemic; for these and other reasons many points of importance and interest were unfortunately not determined.

Bacteriological Examination.—The microscopic and bacteriological investigation was limited to the examination of six specimens of intestinal discharges, four from the S. family and two from the C. cases. Three of these six specimens were from fatal cases, two from the S. cases and one from the fatal case in the C. family. The investigation also extended to the examination of five specimens of the drinking water, one specimen from each of the three wells at the C. habitation and two from the only well at the S. house.

A careful search for amœbæ was made in the specimens of intestinal discharges, with negative results in every instance. Amœbic dysentery is practically unknown in this section of the country.

Intestinal Discharges.—Cover-slip preparations stained by aniline gentian violet proved the predominating organism to be a bacillus of moderate length and thickness, showing little disposition toward any definite arrangement. A few other forms of bacteria were also present and resembled such micro-organisms as are usually observed in fæcal material. Epithelial cells, and in some cases some blood and granular debris, were likewise observed.

Gelatin and agar plates were made from all these specimens. Both sets showed an abundant growth of variously sized colonies which in the case of the gelatin plates always consisted almost exclusively of liquefiers. In three or four days the plates showed a greenish color throughout the medium. Further investigation demonstrated that the liquefying colonies consisted of a bacillus usually of the same morphological appearance when taken from different colonies in the same plate, but differing frequently in its morphology in different plates. Occasionally a discrete colony of a dirty white, brown or orange color was detected in the plates. A number of separate varieties of organisms was isolated: (1) a bacillus producing a green-colored pigment and in all the plates the most abundant organism; (2) another bacillus, a liquefier, but apparently producing no pigment, and (3) a non-liquefying and non-pigment-producing bacillus. Both of these latter varieties morphologically resembled, more or less, the predominating bacillus in the plates. A few saprophytic bacteria were also isolated.

The green pigment produced behaves in the following manner: In cover-slip preparations the organism proved to be a bacillus quite variable in its morphology when obtained from different media, and sometimes upon the same kind of media. The active motility of this organism was usually well observed in 20 to 30-hour bouillon cultures.

Agar-agar. Profuse, moist, greenish white growth along inoculation tract. After the second day the medium is diffusely colored with a more or less vivid green fluorescence.—*Potato.* Rust-brown, usually profuse growth. The chameleon phenomenon of P. Ernst was obtained.—*Bouillon.* Uniform turbidity with production of a slightly green film as culture becomes older.—*Dunham peptone solution.* Turbidity. Test for indol positive.—*Litmus-milk.* Acidification with precipitation of casein.—*Gelatin stab.* Liquefaction of gelatin with development of greenish color in gelatin.

Pyocyanine crystals were successfully isolated from various cultures by the method recommended by Charrin.

Animal experiments were made with this organism. 0.5 cc. of a 26-hour bouillon culture injected subcutaneously into a rabbit resulted in the production of a large inflammatory infiltration about the seat of inoculation, with subsequent extensive sloughing of the superficial tissue. The animal survived. A similar dose was injected intravenously into a rabbit, and produced stupor and later death in about 30 hours. The autopsy showed congestion of lungs, kidneys, ecchymoses in liver and punctiform hæmorrhages in intestinal mucosa with congestion. The bacillus originally inoculated was isolated from the blood and viscera. The cultures used in the above inoculations were both derived from specimens of the intestinal dejecta. A guinea-pig received 1 cc. of a 20-hour bouillon culture (obtained from specimen of water) intraperitoneally. Stupor and slight diarrhœa and death on second day. Postmortem examinations showed small hæmorrhages into peritoneum, pericardium and intestinal walls; congestion of latter as well as of kidneys; spleen swollen. The bacillus originally inoculated was cultivated from the abdominal viscera, but not from the blood.

Diagnosis.—Bacillus pyocyaneus (Gessard).

The extreme degree of variation noted in the morphology of the bacillus pyocyaneus has frequently been observed. This polymorphism was carefully studied by Charrin and Guignard* with particular reference to the conditions favoring its production.

The non-pigment-producing liquefier in cover-slips is seen to consist of a bacillus of variable size. This is also an organism of considerable motility.

Agar-agar. Moist, gray-white growth. Offensive odor.—*Gelatin stab.* Liquefaction usually well marked in 48 hours.—*Bouillon.* Uniform cloudiness of medium in 20 hours.—*Litmus-milk.* Rapid acidification and precipitation of casein.—*Glucose agar.* Abundant gas production.—*Saccharose agar.* Slight gas production.—*Potato.* Thick dirty-brown growth with strong trimethylamine odor.—*Dunham solution.* Turbid. Test for indol positive.

Diagnosis.—Proteus vulgaris (Hauser).

The third variety isolated was a short, thick bacillus, taking aniline-gentian violet stain well. Motility of the bacillus moderately active in 24-hour old cultures.

Agar-agar. Dirty white, sometimes brownish growth along tract of the needle.—*Bouillon.* Marked turbidity.—*Potato.* Abundant yellowish brown growth which later changes to darker color.—*Dunham solution.*

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* *Académie des Sciences,* 12 Decembre, 1887.

Cloudiness. Indol reaction.—*Litmus-milk.* Acid reaction, precipitation of casein.—*Glucose agar.* Slight gas production.—*Saccharose agar.* Slight gas production.—*Gelatin stab.* No liquefaction.

Diagnosis.—Bacillus coli communis.

The bacillus pyocyaneus was present in almost pure culture in every specimen of the intestinal discharges examined, the bacillus coli communis in two specimens in very small numbers, and proteus vulgaris in three specimens, once in association with pyocyaneus and colon bacillus and twice with pyocyaneus alone. Associated with these three varieties was an exceedingly small number of colonies of common intestinal saprophytes.

The same methods of investigation were extended to the examination of the five samples of well water. The plates contained abundant numbers of liquefying colonies elaborating a green pigment, and further study demonstrated the colonies to be those of the bacillus pyocyaneus. In a specimen from one of the wells not used for drinking purposes by the C. family, a few colonies of the proteus vulgaris were isolated. All of these specimens in addition contained some common saprophytic water micro-organisms.

The probability of the causative relation of the bacillus pyocyaneus to the epidemic of dysentery is further strengthened by the fact that it is not a normal intestinal saprophyte. This has been well demonstrated by several investigators, and, indeed, its presence in the intestinal canal is not only very uncommon, but should be regarded with extreme suspicion, as was pointed out by Kossel.

Calmette, as a preliminary study to his investigations upon enterocolitis prevalent in Cochin-China, examined the stools of a large number of normal individuals in order to ascertain the exact nature of the normal microbic intestinal flora. In none of these bacteriological researches did he isolate the bacillus pyocyaneus, and therefore concluded that its presence in the intestinal tract cannot be regarded as normal. Kossel, from the results of numerous examinations of normal and pathological stools, confirmed the conclusions of Calmette. The presence of the organism in pathological conditions of the intestinal canal is by no means common. W. D. Booker,* in his work upon the summer diarrhœas of infants, found it only in occasional instances. Such an opinion receives additional confirmation from Krannhals,* who, in a bacteriological investigation of some 60 various intestinal disorders, only succeeded in isolating it once from an intestinal abscess.

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* *Johns Hopkins Hospital Reports.* Vol. vi.

The literature of dysentery referable to the bacillus pyocyaneus is extremely scanty. Outside of the researches of Calmette and Maggiora nothing can be found except isolated instances either of general or localized infection in which the intestinal symptoms were prominent, but generally associated with others of various kinds.

Calmette in the dysentery of Cochin-China found the bacillus pyocyaneus to be present in 15 cases out of 16 investigated, in the intestinal contents and ulcers in such abundance that it was practically the only bacterium which grew in the plates. From some of these cases it was also obtained from the blood in pure culture. The frequent association of the streptococcus with the bacillus pyocyaneus in the intestinal contents caused Calmette to attribute to the coccus the role of a predisposing factor in the production of the pyocyanic infection. Recently E. Phisalix† has published the results of experimental work which apparently demonstrates the increased susceptibility to pyocyaneus infection when associated with the staphylococcus pyogenes aureus. This is, however, true of many infections with other organisms.

Clinical evidence certainly shows that such infection is relatively more common as a secondary complication, and furthermore that age is a predominating factor. Young children in whom infection by pyogenic or other organisms is common, and where resistance is low, are those in whom pyocyaneus infection is most apt to occur. Infections with this micro-organism are not, however, confined to children, as many authors are inclined to believe, but are also relatively frequent in adults.

Patients suffering from the Cochin-China dysentery are affected with gastric disturbances, abdominal pains and frequent alvine evacuations, while not uncommonly a green discoloration of the intestinal discharges is seen. The symptoms presented by the cases reported in this paper were in many respects not unlike those studied by Calmette.

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* Loc. cit.

† *Compt. rend. Soc. de biol.*, Feb. 27, 1897.

In 1891 Maggiora,* in the dejections from 20 cases of epidemic dysentery which existed in the north of Italy, found colon bacilli and extremely virulent cultures of the bacillus pyocyaneus. Thiercelin,† and Williams and Cameron‡ found them in the pyretic and other forms of the gastro-enteritis of infants, as did also Lange.§

Of the small number of isolated cases of pyocyaneus infection which have been published from time to time, the following presented symptoms which were chiefly referable to the intestinal tract. Oettinger's¶ case was a young man who on the twentieth day of his convalescence from typhoid fever had what was apparently a relapse, but skin vesicles soon appeared and diarrhœa became very marked. Previous to the appearance of the vesicles constipation had existed. The pyocyaneus was obtained in pure culture from the vesicles and urine. Kossel observed in a four weeks old infant vomiting, diarrhœa and green stools. The patient died and cultures contained the bacillus in practically pure growth in the blood and intestinal discharges. Krannhals,|| Finkelstein** and Triboulet†† have reported similar cases in which diarrhœal symptoms were prominent and the color of the stools was more or less markedly green.

The etiological relations of the bacillus pyocyaneus to epidemic dysentery is probably very infrequent, for Krannhals in the examination of 40 cases failed to find the bacillus in a single instance. A number of other investigators who have examined cases of dysentery have likewise failed to find it.

The bacteriological findings in the epidemic which occurred at Hartwick and which was studied by us, demonstrated the pres-

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* *Centralb. f. Bact.*, 1891.

† Thèse de Paris. 1895.

‡ *Journal of Pathology and Bact.*, 1896.

§ See Heubner in Pentzold-Stintzing's Handbuch der Therap., vol. iv, p. 169.

¶ Loc. cit.

|| Loc. cit.

** Loc. cit.

†† Loc. cit.

ence of the bacillus pyocyaneus constantly and in abundant numbers in every specimen of water and intestinal discharges. Furthermore, animal experiments showed this organism to be possessed of a considerable degree of virulence. Attention must also be called to the fact that the bacillus was not only the sole micro-organism present in great numbers in every case, but also that it was the only organism invariably found in the water.

The proteus vulgaris was isolated from two specimens of the intestinal discharges and once from a sample of water from a well not utilized for drinking purposes at the time of or previous to the outbreak of the epidemic. The bacillus coli communis was in no instance isolated from the water and only twice from the intestinal specimens.

The constancy of the presence of the bacillus pyocyaneus in both the discharges and drinking water, the numbers in which it was found and the virulence of the cultures furnish evidence of such a nature as to leave little doubt in the author's mind as to the etiological relation of the bacillus pyocyaneus in the production of this epidemic of dysentery. This view, it seems to me, is strengthened by the similar observations of Calmette and Maggiora, and the not infrequent production of diarrhœas in children by this organism.

A HISTOLOGICAL STUDY OF TYPHOID FEVER.*

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PLATES LIV-LXI.

The object of this paper is to throw some light on the primary, essential lesion of typhoid fever, and also on certain secondary lesions which result therefrom.

Little or nothing can be added to the classical descriptions by Hoffmann, Orth and others of the gross lesions occurring in typhoid fever. On the other hand, the descriptions of the histology of these lesions, of the cell changes in the various organs and of their relation to the general pathological process peculiar to typhoid fever, leave much to be desired.

The essential points which have been discovered in the histology of typhoid lesions can be summed up briefly.

In 1848 Virchow believed and taught that the swelling of the intestinal lymphoid tissue and of the mesenteric lymph nodes was due to a simple exudative process. Later, however, he convinced himself that the swelling was due entirely to hyperplasia of the cells of the lymphoid tissue, and vigorously opposed the view held by the Vienna school that it was caused by an albuminous exudation. He also considered that the swelling of the spleen was essentially hyperplastic in origin.

In 1857 Friedreich called attention to certain small circumscribed collections of cells in the liver and kidneys from a case of typhoid fever.

In 1860 and 1861 Wagner described more fully similar small grayish nodules which were found in the liver and kidneys, and subperitoneally over the ileum, cæcum and appendix in several cases of typhoid fever. Since his time the term “lymphoid nodules” has been applied to these collections of cells.

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* Read before the Philadelphia Pathological Society, April 28, 1898.

In 1861 Billroth and Grohe, working independently, described certain very large multinucleated cells occurring in the mesenteric lymph nodes in typhoid fever. Both regarded them as examples of endogenous cell formation.

In 1862 Billroth called attention to the presence of these same large cells in the blood-vessels of the spleen and in the portal circulation. He traced the cells into the liver and concluded, after measurements both of the cells and of the liver capillaries, that it was possible for the cells to pass through the liver and reach the lungs. He suggested the possibility that the cellular foci of the liver described by Friedreich and Wagner might be due to emboli of these cells.

In 1869 Hoffmann observed these same large multinucleated cells in Peyer's patches and in the solitary follicles of the intestine in early cases of typhoid fever.

In 1875 Klein described them again in the intestine and mesenteric lymph nodes, with wonderful accuracy considering the technical disadvantages under which he labored, but he drew wrong conclusions from what he saw. He called attention to the large cells filled with red blood globules present in the spleen in typhoid fever.

In 1895 Reed claimed that the term "lymphoid nodules" applied to the focal lesions in the liver was incorrect, because these lesions were really areas of focal necrosis of liver cells invaded by polymorphonuclear leucocytes.

In recent years the histology of typhoid fever has been neglected for the typhoid bacillus and the serum reaction. Text-books on pathology pay little attention to the microscopic appearances of the early lesions of typhoid fever. The best, that of Orth, says that the essential cause of the swelling or new growth in the intestine is an accumulation in and around the lymph nodules of large cells, only in part of the character of ordinary lymph cells, and often containing many nuclei and occasionally red blood globules.

The latest views in regard to typhoid fever are those expressed by Ribbert in his Lehrbuch der pathologischen Histologie, published in 1896. He claims that the primary swelling in the intestine and mesenteric lymph nodes is due to the typhoid bacilli getting into the lymph sinuses and causing an acute inflammatory exudation of serum and leucocytes into the outer portion of the lymph nodules and the adjoining tissue. After a certain length of time the exudation disappears and is followed by an intense proliferation and desquamation of the endothelial cells lining the reticulum of the lymph spaces. These cells are occasionally phagocytic. The real lymphoid nodules of the lymphatic apparatus, according to him, play no part in the process. Later, necrosis and sloughing of the newly-formed tissue occur, and repair by granulation tissue follows from the base of the ulcer.

A pathological lesion can be fully understood only when every step in its development, and especially its beginning, can be followed and studied. Where lesions can be produced experimentally this is a comparatively simple matter. For the lesions of typhoid fever, however, we are dependent on material obtained from post-mortem examinations, and we rarely have the fortunate combination of an early case of the disease and an early post-mortem examination, an absolute necessity for the perfect study of the lesions of the intestine. Other requisites are exact fixation of the nuclei, protoplasm and red blood globules, and a staining method which shall clearly differentiate the various tissue elements met with. For the fixation of the tissues Zenker's fluid has been found to give by far the best results, while for staining eosin followed by Unna's alkaline methylene-blue solution has afforded the clearest pictures.

During the past year I have been able to collect and to study histologically 19 cases of typhoid fever, 11 from the Boston City Hospital, 3 from the Massachusetts General Hospital through the kindness of Dr. J. H. Wright, and 5 from other sources. The duration of the disease in the cases examined varied from 10 days to 4 weeks.

The starting point of this investigation into the histogenesis of typhoid fever was a case of the disease in a girl eleven years old, who died at the Children's Hospital on the tenth day after the appearance of the first symptoms. The post-mortem examination was made one hour after death. The Peyer's patches and the solitary lymph nodules of the intestine showed marked swelling, and occasionally small yellowish foci due to necrosis, especially at the lower end of the ileum, but no macroscopic evidence of ulceration. The mesenteric lymph nodes were much enlarged and hyperæmic, and the spleen was considerably larger than normal, firm to the touch and dark in color. Portions of the intestine were put at once, without washing the surface, into alcohol, Zenker's and Orth's fluids, and sections of the various organs were preserved in the same fixatives.

Although no other case was obtained so early in the disease or so soon after death, yet it was possible in seven other instances to trace the whole development of the intestinal lesions, partly because the cell changes in typhoid fever are comparatively slow, and partly because all of the swollen Peyer's patches usually do not undergo necrosis and sloughing.

In the following histological descriptions of the various lesions in typhoid fever the different organs, beginning with the intestine, will be considered separately.

INTESTINE.

The lymph nodules of the intestine consist of masses of lymphoid cells lying in the meshes of a delicate connective-tissue reticulum. Lining this reticulum are certain large cells with vesicular, lightly staining nuclei, usually looked upon as the cells of the reticulum, but regarded by Ribbert and some others as endothelial cells intimately adherent to the reticulum, which is said to have a few spindle-shaped, deeply staining nuclei of its own. Owing to the close relationship between endothelial and connective-tissue cells embryologically and pathologically, and to the fact that the endothelium of newly-formed blood-vessels has been shown in the organization of thrombi to produce connective tissue, it is doubtful if these two varieties of cells can ever be sharply differentiated. In the lesions to be described, however, the large cells of the reticulum of the lymphoid tissue behave in all respects like the endothelial cells of the lymph and blood-vessels. This fact might be used as an argument, therefore, in favor of their being endothelial rather than connective-tissue cells. In the following pages these cells will be spoken of as endothelial cells.

Under the influence of the toxic product of the typhoid bacilli these larger endothelial cells increase in number (Plate LV, Fig. 4). The new cells are epithelial in character, with round or oval, vesicular nuclei surrounded by a considerable amount of protoplasm, which is always sharply outlined and is stained pink by the staining method employed. The proliferation of these cells may take place diffusely throughout the lymph nodule (Plate LIV, Fig. 2), but may also start in the centre (Plate LIV, Fig. 1) or at the periphery.

The cells increase not only in number but also in size, and soon begin to incorporate (Plate LV, Fig. 5) and to digest the neighboring lymphoid cells. In a short time the only lymphoid cells left are those in that part of the lymph nodule where the endothelial cells have not proliferated. As these cells increase in size the nuclei lie eccentrically, usually near the periphery, and almost invariably become irregular in shape, either curved or variously indented. The cells are generally spherical in shape, but through pressure are often rendered irregular or elongated. Only rarely is a form seen that suggests amœboid movements on the part of these cells which probably do not change their relative positions, although they must have some power of motion in order to incorporate the cells found within them. Occasionally these large cells have two or more nuclei of their own.

These newly-formed cells all show to a marked degree the property of phagocytosis. Often 10 to 20 or more lymphoid cells in various stages of digestion will be found within one of the large cells (Plate LV, Fig. 6). As the lymphoid cells undergo digestion the nuclei shrink a little, become homogeneous in appearance and stain very deeply with nuclear stains. They usually dissolve irregularly and may become hollowed out into crescentic forms or break into fragments. As a rule, the incorporated cells can be seen to lie within digestive vacuoles. Occasionally red blood globules, more rarely plasma cells and polymorphonuclear leucocytes, are taken up by these large cells and digested in the same manner as the lymphoid cells. Outside of the lymphoid nodules, the less clearly defined lymphoid tissue shows an irregular, more or less diffuse formation of large cells with properties similar to those already described. The same cells are found in large numbers in the dense fibrous tissue of the submucosa, both beneath the patches of Peyer and the solitary lymph nodules, and also for some little distance peripherally beyond the limits of the lymphoid tissue. The cells are usually elongated, owing to pressure, and show marked phagocytic properties.

Orth has called attention to the swelling of the mucous membrane of the intestine, especially in the lower part of the ileum, in typhoid fever. Microscopic examination shows that the swelling is due largely to the same cell changes that are found in the lymph nodules. They are most marked in the mucous membrane covering and adjoining the patches of Peyer, but also occur to a moderate degree everywhere in the mucous membrane of the lower part of the ileum. The changes are always found more marked between the glands of Lieberkühn (Plate LV, Fig. 3) than in the villi, and consist of proliferation of the endothelial cells in the fibrillar reticular connective tissue, the meshes of which in this situation contain usually more plasma than lymphoid cells and also a fairly large number of eosinophiles. The newly-formed cells do not differ in character from those already described.

Proliferation of the endothelial cells is shown by mitosis in all of the situations in which these cells are found (Plate LV, Figs. 3 and 4). While not abundant, it is sufficiently frequent to account for all of the new cells formed.

Besides these cells derived by proliferation from endothelial cells and characterized by marked phagocytic properties, there are certain other cell changes which have to be taken into consideration. The epithelial cells in the glands of Lieberkühn are in a state of very active proliferation, as shown by numerous mitotic figures present in every gland. How much more abundant, if at all, the cell division is than in the normal intestine it is impossible to say. Occasionally a gland of Lieberkühn is dilated and filled with polymorphonuclear leucocytes. Mitosis is also very frequent in the lymphoid cells remaining in the lymphoid tissue and in the mucous membrane. The mitotic figures can be differentiated from those of the endothelial cells because they are smaller and by the fact that their protoplasm is very granular and stains deeply with methylene-blue. Many of the lymphoid cells have changed or are changing into plasma cells characterized by small, round, coarsely granular, deeply staining, eccentrically situated nuclei and finely granular protoplasm which stains deeply with methylene-blue. Besides the lymphoid and plasma cells originally in the tissues or derived from them, others are added to them by emigration from the blood-vessels (Plate LVI, Fig. 14), Large multinucleated plasma cells are found occasionally. Irregularity of shape, due to amœboid movements, is shown by many of the lymphoid and plasma cells.

In the muscular coats of the intestine the cellular increase is most noticeable along the lymphatic vessels. The cells consist in part of infiltrating lymphoid and plasma cells, but principally of large phagocytic cells in the lymph vessels and spaces around and near the blood-vessels. Similar cells are also found between the smooth muscle fibres and are generally elongated in shape in consequence of pressure. Mitotic figures can occasionally be found, and the cells often occur in small clumps.

Subperitoneal collections of cells are usually found only beneath the patches of Peyer. As in the muscular coats, they consist of collections of phagocytic cells derived by proliferation from the endothelial cells around the blood-vessels. Occasionally fairly large clumps of cells are formed.

LYMPHATICS.

The terminal lymphatics of the mucous membrane of the intestine by the tenth day of the disease are distended and filled with large phagocytic cells derived from the lining endothelium (Plates LV, Fig. 3, and LVI, Fig. 8). Along with them is usually a small number of lymphoid and plasma cells. In consequence of their distention with cells, it is easy to follow the lymphatic vessels into and all through the submucosa (Plate LV, Fig. 7). The cellular contents vary to some extent in the relative number of phagocytic and lymphoid cells, but as a rule the large cells predominate.

BLOOD-VESSELS.

Microscopic examination of the distended blood-vessels of the mucous membrane and submucosa shows a surprising absence of polymorphonuclear leucocytes. In their stead are occasional lymphoid and plasma cells, which not infrequently divide by mitosis in the blood current, and very numerous, large cells with lightly staining, vesicular, curved or indented nuclei (Plate LVI, Fig. 10). Many of these cells are phagocytic and contain red blood globules, less often lymphoid and plasma cells or polymorphonuclear leucocytes.

The number of cell inclusions is usually small and is limited chiefly to red blood globules. The large cells are evidently derived from the vessel walls, because examination of the lining endothelium of the capillaries and veins of the mucosa and submucosa shows mitotic figures to be quite numerous (Plate LVI, Figs. 9 and 12). During mitosis the cells are usually adherent to the wall but may be somewhat loosened. Much more rarely these large cells divide by mitosis when free in the blood stream.

The lesions thus far described are proliferative in character and probably mark the limit reached by the typhoid process in many of the agminated and solitary lymph nodules in mild cases of the disease. Even in severer cases some of the patches of Peyer do not undergo necrosis with sloughing and ulcer formation. Instead, the large newly-formed phagocytic cells rapidly undergo fatty degeneration and disappear. When the typhoid process has been more severe, however, certain changes take place which lead to obstruction of the lymphatics and to the obliteration of many of the blood-vessels, thereby causing necrosis of the tissue in which they lie.

Virchow thinks that the necrosis is due to a form of caseation much like that occurring in tuberculosis, while Orth is of the opinion that the necrosis is due to pressure exerted on the blood-vessels in consequence of the great hyperplasia of the cells. Careful study leads me to the view that possibly some of the capillaries may become occluded by proliferation of their endothelial cells, but the truth of this view or of Orth's is difficult to demonstrate. By far the most common method of occlusion of the blood-vessels, and perhaps the only one, takes place in the following way:

The first regressive changes in the intestinal lesions of typhoid fever are shown in the large phagocytic cells in the lymphatic vessels. The cells degenerate and an abundance of fibrin soon binds them together. The outlines of the cells disappear and the nuclei become more irregular than before. In this way thrombi are formed which occlude the vessels, so that it is difficult to believe that much, if any, circulation can take place in them under such circumstances.

Beneath the lining endothelium of the large and smaller veins, much more rarely of the larger lymphatic vessels, accumulations of cells take place in what must be a lymph vessel or lymph space. The cells consist in part of lymphoid and plasma cells, in part of large phagocytic cells derived by proliferation from the cells lining this lymph space (Plate LVII, Fig. 15). The cell accumulations, which project more or less into the lumina of the veins, may be large or small, focal in character or spreading out for some distance around or along the vessels. Necrosis of the large phagocytic cells takes place very frequently in these collections of cells beneath the endothelium and furnishes the starting point for a very abundant formation of fibrin which often extends for some distance along the vessel (Plate LVII, Fig. 16). The nuclei of the large cells undergoing necrosis usually become more irregular in shape than normally before they lose the property of staining and disappear. The lining endothelium of the vessel is generally preserved intact (Plate LVII, Fig. 19), at least for a time, over such a collection of cells, but often by necrosis becomes the starting point for the formation of fibrin which reaches out into the lumen and by extending along it may divide it into two or more channels. The cells in the blood current get caught in this fibrin and thrombi are soon formed which completely occlude the vessel (Plate LVII, Fig. 17). From these primary foci the fibrin formation extends along the vessels and into the capillaries, thereby occluding them. So far as can be ascertained by a careful study of serial sections, these masses of fibrin, which are often very numerous beneath the lining endothelium of veins before any other regressive changes have taken place anywhere in a Peyer's patch, always start from the protoplasm of degenerating phagocytic cells. The fact that these large cells do form beneath the lining endothelium of the veins is best seen in the larger vessels of the spleen (Plate LXI, Fig. 34) and liver. The areas thus deprived of their blood supply by occlusion of the veins and capillaries soon show evidence of undergoing necrosis. The outlines of the cells become indistinct and more or less fused, owing to the formation of fibrin which is not always very abundant. The nuclei become even more irregular in shape than normally and finally disappear. The included, partially digested cells break up into fine particles which after a while refuse to stain.

The inflammatory exudation which takes place in consequence of the necrosis of the tissue consists almost wholly of serum in which abundant fibrin forms. Polymorphonuclear leucocytes are rarely found. The exudation, as is usual in the intestine, collects in greatest quantity in the deeper layers of the submucosa. The earliest necroses seem to take place generally in the lymph nodules which were first affected. The mucous membrane also, which is very thin over them, quickly undergoes necrosis. The upper portions of these necroses soon macerate and slough off. In this way small superficial ulcerations are formed, along the edges of which numerous bacteria quickly collect. Sometimes, however, the mucous membrane over a Peyer's patch is fairly well preserved when necrosis in the submucosa is already well advanced. In the small necroses polymorphonuclear leucocytes are very uncommon, but in the large necroses they usually occur in considerable numbers, not along the edge of the necrotic tissue, but nearer the surface where the various bacteria of the intestinal tract are invading the necrotic tissue. The irregularity of the necrosis and ulceration of a Peyer's patch depends, of course, on the fact that all of the veins usually do not become occluded. Even when the necrosis is quite marked there is generally a little tissue immediately around the arteries supplying the affected areas which does not undergo necrosis, at least at first, because the arteries, unlike the veins, do not become thrombosed.

MESENTERIC LYMPH NODES.

The lesions occurring in the mesenteric lymph nodes do not differ in character from those found in the lymph nodules of the intestine. In fact the mesenteric lymph nodes offer the very best tissue for studying the essential lesion of the typhoid process in all its stages, because they are not so freely exposed to invasion by various organisms as is the intestine the moment its lining epithelium is injured.

In the lymph nodes the lymph sinuses are always more affected than the nodules and cords of lymphoid cells. They become widely dilated and filled with large phagocytic cells containing mainly lymphoid and plasma cells, but also occasionally red blood globules. Besides the large cells, numerous lymphoid and plasma cells are also present in the sinuses. The large cells are unquestionably derived in part, if not entirely, from the endothelial cells lining the loose reticulum of the sinuses.

In the lymphoid tissue between the sinuses a proliferation of the large cells lining the reticulum takes place just as in the intestine. Sometimes it is most marked centrally, sometimes diffusely or peripherally. Mitosis of the endothelial cells of the reticulum of the sinuses and lymphatic tissue is fairly frequent. Necrosis in the lymph nodes, when it occurs, is always most marked and most extensive in the lymph sinuses. An abundant formation of fibrin takes place, binding the cells together. The nuclei of the large cells become very irregular in shape and finally disappear.

In the lymph nodules the necrosis seems to depend largely on the same lesions of the blood-vessels that are found in the intestine, namely, collections of phagocytic and lymphoid cells beneath the lining endothelium, necrosis of the large cells and fibrin formation with extension into the vessel and occlusion of the lumen.

The typhoid process often extends outside of the lymph nodes into the adjoining fat tissue, where many large phagocytic and plasma cells are found between the fat cells. These areas also may become necrotic.

In many cases some at least of the lymph nodes do not undergo necrosis; instead, resolution takes place; the cells rapidly show signs of fatty degeneration and disappear. Apparently resolution can occur even when fairly extensive necrosis with fibrin formation exists in the lymph sinuses. What the exact changes are which take place when a whole lymph node becomes necrotic I have not been able to follow. Certain it is, however, that the necrotic tissue ordinarily shows very little or no infiltration with polymorphonuclear leucocytes.

SPLEEN.

I wish to speak next of the spleen, because the cell changes which take place in it have an important bearing on the lesions of the liver.

As a rule the lymph nodules of the spleen do not show any marked change in typhoid fever. In certain cases, however, some or all of the nodules show to a greater or less extent the same formation of phagocytic cells from the endothelial cells of the reticulum that is found in the lymphoid tissue of the intestine.

The most marked changes of the spleen are those found in the blood-vessels and spaces of the pulp. They are much dilated and filled with large phagocytic cells containing principally red blood globules, usually in very large numbers, but also lymphoid and plasma cells and polymorphonuclear leucocytes. These phagocytic cells are derived chiefly or entirely by proliferation and desquamation from the endothelial cells lining the vessels. Occasionally mitosis takes place in the cells lying free in the blood. Sometimes the blood-vessels and spaces become occluded by masses of these large cells, which degenerate and become bound together by a meshwork of fibrin.

Besides this general proliferative process we get, as in the intestine, numerous plasma cells in the veins and in the splenic pulp. Many of them show amœboid shapes and not infrequently mitosis.

In some of the splenic veins, especially the larger ones, the lining endothelium is lifted up by masses of large phagocytic cells, and of lymphoid and plasma cells (Plate LXI, Fig. 34). In places these cells degenerate and fibrin forms between and around them.

LIVER.

Two varieties of focal lesions, to both of which probably the term "lymphoid nodule" was applied originally, are often found in the liver in typhoid fever. One is confined to the small amount of connective tissue around the portal vessels, that is, it lies outside of the lobules and in no immediate relation to the liver cells; the other may occur anywhere within the lobule among the liver cells. The two forms of lesion often adjoin each other. It is important to separate them sharply because they arise in different ways.

The lesion around the portal vessels consists to some extent of an increase of lymphoid and plasma cells, due in part to proliferation, in part, probably, to immigration. The chief part of the lesion, however, is formed of large cells with phagocytic properties derived by proliferation from the endothelial cells around the portal vessels. The phagocytic cells do not differ from those found in the intestinal lesions and in the mesenteric lymph nodes, except that the cell inclusions are not so numerous and are confined almost entirely to lymphoid cells. Very rarely a giant cell is formed in this situation, apparently by fusion of the endothelial and other cells in a lymph vessel.

In order to understand the origin of the second form of focal lesion in the liver—that occurring somewhere within the lobule among the liver cells—it is necessary to refer to the lesions of the intestine and spleen. In these organs we found that fairly abundant mitosis of the endothelial cells of the capillaries and veins took place, that these cells desquamated into the blood current, and in the intestine could be followed into the large veins of the submucosa. They were distinguished from the nucleated cells ordinarily found in the blood by their size, the irregular shape and eccentric position of their nuclei, and especially by their marked phagocytic properties. The last feature was most prominent in the cells in the veins and blood spaces of the spleen, where the circulation is probably slower. From these organs many of the large cells are carried directly to the liver. Billroth observed large multinucleated cells in the splenic and portal veins in 1861 and traced them to the liver. As already mentioned, he thought they might be the source of the lymphoid nodules in the liver described by Wagner. He does not seem to have recognized the fact that the large cells often contained red blood globules.

Examination of the livers of the cases that came to post-mortem examination within the first two weeks of the disease shows in many of the branches of the portal vein, numerous large phagocytic cells containing usually many red blood globules or undigested portions of them, less frequently lymphoid cells, and rarely polymorphonuclear leucocytes (Plate LVIII, Fig. 21). These same cells are found in the capillaries all through the liver, often closely crowded together. Mitosis may take place in them either in the large vessels or in the capillaries (Plate LVIII, Fig. 23). The cells often assume irregular and elongated shapes, evidently from the pressure to which they are subjected in passing through the narrow vessels. Occasionally these cells undergo necrosis, as is shown by the fact that the various stages in the cell changes leading up to the picture presented by complete necrosis can be followed, and in these earlier stages the cells can be definitely recognized by the inclusions in the protoplasm.

Occasionally some of these large cells stick fast in the capillaries. At least four causes favor this condition, viz.: the general swelling of the liver cells from parenchymatous degeneration leading to narrowing of the capillaries, the very large size of many of these phagocytic cells in consequence of the great number of their inclusions, the swelling and phagocytosis of the endothelial cells lining the liver capillaries, and finally, mitosis of the capillary endothelium (Plate LVIII, Fig. 20).

When by obstruction of a capillary a focus of cells is formed, other cells are added to it, partly, it would seem, from mechanical causes owing to interference with the circulation at such a point, partly from cell division as is shown by mitosis, so that soon a number of liver cells are completely surrounded by these large cells (Plate LVIII, Figs. 22 and 25). Then the included liver cells, presumably on account of the interference with their nutrition, quickly undergo necrosis, as is shown by the deeper stain of their protoplasm with eosin (Plate LIX, Fig. 27). The nuclei at first stain deeply (Plate LIX, Fig. 27), then they and the protoplasm break down into granular detritus and disappear (Plate LX, Fig. 29). Sometimes the cells which block up the capillaries undergo necrosis before the liver cells (Plate LX, Fig. 28).

Pressure on the surrounding liver cells, making them narrow and elongated, is often exerted peripherally by these clumps of cells owing to the proliferation in them. It is possible that some of the foci in the liver may arise solely from the proliferation of the endothelial cells lining a capillary. The cells within the focal areas usually do not contain many inclusions, probably because those which they may have taken up in the circulating blood have been more or less digested, and because the newly-formed cells in the foci do not have the same opportunity for getting hold of other cells. Mingled with these large cells in the focal areas are often a few lymphoid and plasma cells, and occasionally red blood globules.

Sooner or later necrosis of these large cells in the focal areas takes place, possibly not always, for it is presumable that at least the smaller collections of cells may undergo fatty degeneration and disappear as in the intestine and mesenteric lymph nodes. The necrosis begins in the centres of the areas; the protoplasm loses its sharpness of outline, fibrin usually appears between the cells and binds them together (Plate LIX, Fig. 26), the nuclei of the large cells become still more irregular in shape and finally disappear. Invasion of the necrotic areas in the liver with polymorphonuclear leucocytes even as late as the fourth week is infrequent and slight. What becomes of the areas after this time could not be ascertained owing to the lack of appropriate material in which to trace the process.

This form of focal lesion, accidental in its location in a liver lobule and leading to the destruction of a limited number of liver cells, is not confined to typhoid fever, but occurs in other infectious processes. It may also result from cell emboli derived from malignant growths. The same variety of lesion can be produced experimentally in 24 to 48 hours by injecting a sterile suspension of carmine in normal salt solution into a mesenteric vein of a guinea-pig.

There is a form of primary necrosis of liver cells which occurs in many acute infectious processes, and is due apparently to some strong, diffusible toxine. It follows practically the same distribution as that seen in chronic passive congestion, *i. e.* the necroses are confined fairly definitely to the centre of every lobule. The capillaries remain pervious, the necrotic liver cells are quickly invaded by polymorphonuclear leucocytes. It was produced experimentally by injecting a dose of diphtheria toxine sufficiently large to kill a rabbit in three days. The muscles all over the body showed intense fatty degeneration and the centre of every lobule of the liver was necrotic. No example of this type of lesion was found in any of the cases of typhoid fever.

Many of the phagocytic cells pass through the capillaries of the liver and get into the lungs, where they can be found in the larger and smaller blood-vessels and in the capillaries (Plate LXI, Fig. 33). They do not seem to cause any obstruction, but pass along into the general arterial circulation. Sections of the pale clots of blood from the right ventricle of the heart in a case that died on the fourteenth day of the disease showed numerous large phagocytic cells among the normal cells of the blood. Sections of a clot from the left auricle showed them present there also in about equal numbers (Plate LXI, Fig. 32). There is no question, therefore, about these cells getting into the general arterial circulation. Examination of the pale watery fluid in the thoracic duct in this same case showed an occasional large phagocytic cell containing numerous lymphoid cells. It is evident, therefore, that cells also get into the general circulation through the thoracic duct (Plate LXI, Fig. 30). The cells derived from the lymph vessels show almost nothing but lymphoid cells as inclusions, while those that come from the portal circulation contain chiefly red blood globules.

It is difficult to explain why these large cells have not been found in the ordinary blood examinations during the second and third weeks of typhoid fever, when they are most abundant, except on the ground that the dried cover-slip method of examining blood breaks up cells of this type. In 1874 Eichhorst reported finding from 2 to 4 large cells each containing 2 to 5, rarely as many as 7, red blood globules in every drop of blood drawn from the tip of the finger of a typhoid patient. This condition was present for five days during the second week of the disease, after which time the large cells suddenly disappeared. Slight pressure upon them caused these cells to break up and set the globules free. I have found them on careful examination in the blood-vessels of the heart, kidneys and brain. So far as can be judged from the sections of the clots in the heart, the phagocytic cells may form at least from 1 to 2 per cent of the nucleated cells in the circulation.

The kidney is the only organ in which the phagocytic cells in the general circulation seem to give rise to any lesion.

It has been shown by Dr. W. T. Councilman* that when the lymphoid and plasma cells increase in number in the general circulation, they filter out into the veins in the pyramids of the kidneys, and may often be there in great numbers. Under certain conditions these cells emigrate from the vessels and give rise to focal collections of cells. In typhoid fever the large phagocytic cells filter out in the same way and crowd the vessels along with numerous lymphoid and plasma cells. Occasionally the large cells occlude several adjoining blood-vessels, and by cutting off the circulation lead to necrosis of the intervening tubules. To complicate the picture, emigration of the lymphoid and plasma cells also present may take place, and hæmorrhage is not infrequent.

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* *Journal of Experimental Medicine*, iii (1898), 393.

The heart was examined in two cases, but in only one was the examination at all thorough. In both cases occasional small foci of lymphoid and plasma cells were found similar in character to the areas often seen in the heart in diphtheria. Besides this form of lesion, phagocytic cells were found here and there in the lymph spaces, especially beneath the pericardium and along the larger blood-vessels. In some of the lymph vessels they were quite numerous. Occasionally a lymph vessel would be occluded by them and then the adjoining lymph spaces would usually also be crowded with them. In this way foci of considerable size would be formed. In one place the lining endothelium of the endocardium was lifted up by a group of large cells (Plate LVII, Fig. 18). In none of these areas was necrosis found. In one brain a single focal lesion was found, but the tissue had been hardened in alcohol and the character of the cells could not be determined. In two cases an occasional phagocytic cell was found in the lymph spaces of the pia-arachnoid.

Examination of the testicles in two cases showed complete cessation of spermatogenesis. One case showed nothing else. In the other the blood-vessels were dilated. Everywhere in the connective tissue between the tubules were numerous large cells in the lymph vessels and spaces. The cells were large and had irregular nuclei and abundant protoplasm; they were often phagocytic and occasionally showed mitotic figures. Sometimes the large cells were clumped together, apparently in connection with the large lymph vessels. In this same case there were numerous fairly large foci of lymphoid cells with a certain proportion of large phagocytic cells among them. Plasma cells and polymorphonuclear leucocytes were rare. Scattered diffusely in the tissue were numerous Mastzellen.

The bone-marrow from the femur was examined in two adult cases, in both of which it was found quite red at the post-mortem examination. The cell changes closely resembled those in the spleen. In the blood-vessels were many large cells containing red blood globules, much less frequently lymphoid cells and polymorphonuclear leucocytes. The large cells also occurred diffusely in the lymph spaces among the other cells of the bone-marrow and around the fat cells. Occasionally clumps of these phagocytic cells were found undergoing necrosis and bound together by fibrin. So far as could be determined, the areas arise by occlusion of blood and lymph vessels by these large cells.

The bladder examined in one case was negative, while the wall of the gall-bladder was infiltrated with numerous lymphoid and plasma cells, and occasionally showed phagocytic cells.

Summing up the lesions thus far described, we have as the essential lesion of typhoid fever a diffuse proliferation of endothelial cells giving rise to large epithelioid cells characterized by the possession of marked phagocytic properties. This proliferation is most marked in the lymphoid tissue of the intestine, in the mesenteric lymph nodes, in the spleen, bone-marrow and liver, but may also be found to a varying extent in the lymphatic vessels all over the body, as shown by examination of the lymphatics of the heart, lungs, testicles and pia-arachnoid.

In regard to the typhoid bacillus I have very little to say and nothing new to add. It was obtained in cultures from most of the organs of the cases studied. Histologically it was rarely found, but it must be confessed that no exhaustive search for the organism was made. Occasionally the characteristic colonies were found in the mesenteric lymph nodes and in the spleen, but not in any case that came to post-mortem examination very soon after death. In the earliest case, in which the post-mortem examination was made one hour after death, no bacteria were found microscopically except along the edges of the beginning ulcerations of the intestine, and in that situation it is of course impossible to differentiate the typhoid from the colon bacillus. Certain it is that when the typical colonies of the typhoid bacillus are found in the different organs they bear no intimate relation to the lesions present.

It would be exceedingly interesting to know whether the typhoid bacillus gains access to the lymphoid tissue of the intestine before any lesion is produced and before the lining epithelium is injured, or whether the lesion is caused primarily by absorption of a toxic substance eliminated by the bacilli which at first are within the intestinal canal only and which gain an entrance into the tissues later.

Unfortunately the line of experimental work so successfully carried out with the diphtheria bacillus and its toxine seems to be out of the question with the typhoid bacillus owing to the immunity in respect to this organism enjoyed by the lower animals. We have to depend, therefore, as yet, for our views in regard to the typhoid bacillus and the lesions produced by it on such deductions as may be drawn from the study of the gross and histological lesions occurring in man.

The following facts favor the view that the intestinal lesions are due primarily to an absorption of a toxic substance secreted by typhoid bacilli in the lumen of the intestine. The lesions diminish in intensity from the ileo-cæcal valve upwards, and are along the line of absorption. The lesions are diffuse. This characteristic is shown in three ways: (*a*) all of the lymphoid tissue for a given distance is generally, although not equally, affected; we do not get here and there a patch of Peyer swollen while the intervening patches remain normal; (*b*) the whole of each patch of Peyer affected shows about the same degree of swelling; (*c*) the mucous membrane in the lower part of the ileum shows diffuse proliferation. In judging of the above points it is necessary to take early cases in which the lesions are in the stage of swelling, because the ulcers, as already shown, depend on more or less accidental causes, which may lead to ulceration of half of a patch of Peyer while the other half undergoes resolution, or to complete ulceration of one or more patches while the others return to normal conditions.

This view that the intestinal lesions are due primarily and perhaps chiefly or entirely to a toxine originating in the intestinal lumen is favored by the character of the lesions occurring elsewhere in the body. They all lie along the lines of absorption through the lymphatics and of distribution through the circulation; they vary in intensity and are always diffuse; an organ, such as the spleen or a mesenteric lymph node, shows practically the same degree of cell change throughout its whole extent. The focal lesions in the liver and other organs all depend on accidental causes.

If the lesions depend on the immediate presence of the typhoid bacillus, then we have to assume that multiple infections of the lymphoid tissue of the intestines take place within a very short time, extend from the ileo-cæcal valve upward and spread uniformly throughout each patch of tissue affected; also that the organism gets into the circulation and reaches the spleen and other organs in the very beginning of the process.

Ribbert bases his opinion that the typhoid process begins with an acute inflammatory exudation on the fact that he finds among the large cells in early cases a little fibrin and an occasional leucocyte which he regards as the remains of the exudation. In view of the lesions above described, it seems more reasonable to regard this evidence of inflammation as the beginning rather than the end of the inflammatory process which is secondary to degeneration of the proliferated cells. Neither can his view that the newly-formed cells are of a reparative nature be accepted; for these cells do not produce any intercellular substance, or form blood-vessels or connective tissue as endothelial cells do in the organization of thrombi and in other reparative processes; instead they either undergo fatty degeneration as in resolution, or extensive necrosis (for which he gives no explanation), followed by ordinary granulation tissue.

The multinucleated lymphoid cells of the early observers are for the most part the phagocytic cells with their inclusions, produced by the action of the typhoid toxine. A very few of them, however, are multinucleated plasma cells.

The typhoid process in the various organs, especially the intestine, spleen and bone-marrow, is to a slight degree complicated by the formation of plasma cells from lymphoid cells, by proliferation of lymphoid and plasma cells, and by their migration into and out of blood-vessels and in the tissues. These lymphoid elements never reach anything like the number present in cases of diphtheria and rarely give rise by emigration to the focal and diffuse collections of cells so often found in that disease, particularly in the kidneys. It is a question whether their increase is due to the direct action of toxic substances or is to be regarded as reparative in nature and dependent on other causes.

This formation by proliferation of phagocytic cells from endothelial cells is not peculiar to typhoid fever except in regard to location, extent and degree. The fact that toxic substances derived from various sources may produce at least two effects on cells, the one degenerative, the other proliferative, and also that they may act focally or diffusely, seems generally to have been overlooked. The staphylococcus pyogenes aureus is a good example of an organism that produces necrosis focally. The toxine is strong and is but slightly diffusible. The tubercle bacillus on the other hand produces proliferation focally. The diphtheria bacillus acts both focally and diffusely and produces, according to the strength of its toxine, either proliferation or necrosis, or the one process following the other. The typhoid bacillus produces a mild toxine that acts diffusely and causes proliferation.

Taking this view of the action of the typhoid bacillus, that it causes proliferation of endothelial cells by means of a diffusible toxine, how are the cases of meningitis, of pneumonia and of abscess formation in bones, the spleen and other organs, reported to be due to the typhoid bacillus, to be explained?

In the spleen of one of the cases studied there were two yellowish nodules, one the size of a pea, the other of a marble. On section the larger nodule was opaque and yellowish white in one-half, while the other half contained thick pus. The smaller nodule was firm except near the centre, where a little pus was present. Bacteriological examination showed only the typhoid bacillus present both in the abscesses and elsewhere in the spleen. On microscopic examination numerous small areas of large phagocytic cells undergoing necrosis and imbedded in abundant fibrin were found all through the spleen. In the neighborhood of the nodules just mentioned some of the large veins were thrombosed by masses of fibrin and degenerating phagocytic cells. The firm portions of the nodules consisted of necrotic tissue in which in places degenerating phagocytic cells could still be distinguished. The softened portions of the nodules contained polymorphonuclear leucocytes. Typhoid bacilli were present in moderate numbers in the form of colonies in the necrotic tissue, and scattered diffusely in the softened portions. In the latter situation many of them were within the leucocytes. This case suggests that possibly the typhoid bacilli, which happened to be in the necrotic area produced through occlusion of blood-vessels by phagocytic cells, may have found more favorable surroundings for growth than they ordinarily do in living tissue, just as the tubercle bacillus grows most luxuriantly in old cavities of the lung or in softened, broken down lymph nodes. It is also possible that the post-typhoid abscesses of bones may owe their origin through obstructed blood-vessels to primary foci of necrosis, in which, as in the necroses in the spleen, the typhoid bacillus finds favorable surroundings for growth.

One of the cases of typhoid fever was complicated with typical fibrinous pneumonia of the whole of the right lower lobe in the stage of gray hepatization. The post-mortem examination was made in a hurry, at night, three hours after death, and unfortunately cultures of the lung were not made. Microscopic examination of the exudation showed serum, fibrin, fairly numerous polymorphonuclear leucocytes, occasionally lymphoid and plasma cells, rarely an eosinophile. There were also present, however, great numbers of large phagocytic cells with irregular, vesicular nuclei situated peripherally (Plate LXI, Fig. 31). The inclusions consisted almost entirely of polymorphonuclear leucocytes, of which usually one or two, but occasionally several, were within a single cell. More rarely the inclusions consisted of red blood globules. The number of phagocytic cells varied considerably in different parts of the lobe, but in many of the alveoli this was almost the only cell present. Microscopically, pneumococci were present in very small numbers, while a bacillus, morphologically like the typhoid bacillus, occurred more abundantly and usually within leucocytes. The perivascular and peribronchial lymph vessels and spaces contained lymphoid, plasma and phagocytic cells in small numbers. There are of course three possible sources for these phagocytic cells within the alveoli of the lung. They may have emigrated from the blood-vessels or have been derived by proliferation from the epithelium lining the alveoli or from underlying endothelial cells after destruction of the epithelium. The third view seems the preferable one. No evidence of emigration or of active amœboid motion on the part of these cells has ever been observed. On the other hand very numerous mitotic figures were found in large cells lining the alveoli. It is of course impossible to deny that the proliferating cells may be epithelial, but in view of the marked phagocytic properties shown by them, it seems more reasonable to assume that they have the same origin as the other phagocytic cells already described. This is assumption, however, not proof.

The simplest explanation of the presence of these phagocytic cells in such large numbers (for they can be found not infrequently in ordinary cases of pneumonia) is that we have here a case of lobar pneumonia complicated by the action of typhoid bacillus of which the toxine has caused a proliferation of certain cells in the wall of the alveoli. How the typhoid bacilli get into the lung I do not pretend to explain beyond suggesting that they may have been brought from the blood-vessels by the emigrating leucocytes. These two cases, the abscesses in the spleen and the lobar pneumonia, certainly suggest that careful bacteriological and histological study of the various complications of typhoid fever, and especially of cases of meningitis claimed to be due to the typhoid bacillus alone, may throw additional light on the character of the lesions produced by the typhoid bacillus.

In view of the lesions described in the blood-vessels of the intestine, liver and spleen, and beneath the lining endothelium of the heart, it seems fair to suppose that the thrombi which sometimes occur in typhoid fever, rarely in the heart but rather frequently in the veins of the lower extremities, may owe their origin to similar lesions.

Remlinger has recently claimed to have produced typical typhoid lesions in a certain proportion of rabbits and guinea-pigs by feeding them every day on vegetables soaked in bouillon cultures of typhoid bacilli. A series of experiments was carried on to confirm, if possible, these results, but without success, although in one case 30 cc. gradually increased to 100 cc. of a bouillon culture were introduced each day for ten days directly into the stomach of a rabbit by means of a stomach tube. The virulence of the organism used may of course have much to do with the success of such experiments.

By injecting 1.5 cc. of a thirty-hour-old bouillon culture of the typhoid bacillus into the peritoneal cavity of a guinea-pig and gradually increasing the amount every day up to 3 cc. on the fifth day, there were obtained on the sixth day an enlarged spleen in which were many large cells filled with red blood globules, and a number of small yellowish foci irregularly distributed in the liver.

Rabbits and guinea-pigs are, however, poor animals in which to study the possible effects of the typhoid toxine for the reason that under what must be considered normal circumstances many phagocytic cells containing the more or less digested remains of lymphoid cells are present, often in abundance, in the lymphoid tissue of the small and large intestine. Attention was first called to these cells by Flemming. Besides these lesions in the intestine, pigmented cells in the mesenteric lymph glands and spleen, and small areas of necrosis in the liver are not infrequent. They are entirely distinct from lesions caused by coccidia, and strongly suggest an origin due to absorption of toxic substances from the intestinal tract. The lesions in the liver are probably embolic in origin, but focal lesions in the liver of these animals are much more difficult to fathom, partly because the capillaries are very narrow and easily occluded, but principally because it is difficult to get perfect preservation of the various cell elements and of the red blood globules, a difficulty due in part at least to the large amount of glycogen present in the liver cells.

CONCLUSIONS.

The typhoid bacillus produces a mild diffusible toxine, partly within the intestinal tract, partly within the blood and organs of the body.

This toxine produces proliferation of endothelial cells which acquire for a certain length of time malignant properties.

The new-formed cells are epithelioid in character, have irregular, lightly staining, eccentrically situated nuclei, abundant, sharply defined, acidophilic protoplasm, and are characterized by marked phagocytic properties.

These phagocytic cells are produced most abundantly along the line of absorption from the intestinal tract, both in the lymphatic apparatus and in the blood-vessels.

They are also produced by distribution of the toxine through the general circulation, in greatest numbers where the circulation is slowest.

Finally, they are produced all over the body in the lymphatic spaces and vessels by absorption of the toxine eliminated from the blood-vessels.

The swelling of the intestinal lymphoid tissue of the mesenteric lymph nodes, and of the spleen is due almost entirely to the formation of phagocytic cells.

The necrosis of the intestinal lymphoid tissue is accidental in nature and is caused through occlusion of the veins and capillaries by fibrinous thrombi, which owe their origin to degeneration of phagocytic cells beneath the lining endothelium of the vessels.

Two varieties of focal lesions occur in the liver: one consists of the formation of phagocytic cells in the lymph spaces and vessels around the portal vessels under the action of the toxine absorbed by the lymphatics; the other is due to obstruction of liver capillaries by phagocytic cells derived in small part from the lining endothelium of the liver capillaries, but chiefly by embolism through the portal circulation of cells originating from the endothelium of the blood-vessels of the intestine and spleen. The liver cells lying between the occluded capillaries undergo necrosis and disappear. Later the foci of cells degenerate and fibrin forms between them. Invasion with polymorphonuclear leucocytes is rare.

Many of the phagocytic cells pass through the liver and lungs, and get into the general circulation. A few come from the abdominal lymphatics through the thoracic duct.

Focal lesions may arise in the kidneys by occlusion of the veins of the pyramids by emboli of these phagocytic cells.

Focal collections of phagocytic cells may occur in the heart and testicle by occlusion of lymph vessels.

The various sequelæ of typhoid fever deserve more careful bacteriological and histological examination, as shown by the study in one case of abscesses of the spleen where these seemed to arise in previously necrotic tissue, and of a case of pneumonia due to the pneumococcus, but complicated by the presence of the typhoid bacillus, in which great numbers of phagocytic cells were found in the exudation.

The thrombi which occur in the heart and in the veins of the lower extremities in the course of typhoid fever probably owe their origin to the same sort of lesions that cause occlusion of the vessels in the intestine.

Histologically the typhoid process is proliferative and stands in close relationship to tuberculosis, but the lesions are diffuse and bear no intimate relation to the typhoid bacillus, while the tubercular process is focal and stands in the closest relation to the tubercle bacillus.

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DESCRIPTION OF PLATES LIV-LXI.

PLATE LIV.

Fig. 1.—Lymph nodule and adjoining tissue from a patch of Peyer showing the centre of the nodule occupied by large phagocytic cells.

Fig. 2.—A solitary lymph nodule showing diffuse formation of phagocytic cells.

PLATE LV.

Fig. 3.—Mucous membrane of intestine showing diffuse formation of phagocytic cells; also a dilated lacteal filled with phagocytic cells.

Figs. 4, 5 and 6 show details from Fig. 1.

Fig. 4.—From periphery of lymph nodule, showing a large cell of the reticulum in mitosis.

Fig. 5.—From near the periphery of lymph nodule, showing newly-formed cells beginning to incorporate the lymphoid cells.

Fig. 6.—From centre of lymph nodule, showing the newly-formed cells filled with more or less digested lymphoid cells. Several red blood globules have also been incorporated.

Fig. 7.—Lymphatic vessel of mucous membrane containing phagocytic, lymphoid and plasma cells.

PLATE LVI.

Fig. 8.—Terminal lymphatic (lacteal) of mucous membrane dilated and filled with phagocytic cells.

Fig. 9.—Mitosis of endothelial cell of capillary in mucous membrane.

Fig. 10.—Phagocytic cells in large vein of submucosa.

Fig. 11.—Plasma cell in mitosis inside phagocytic cell in a large vein in submucosa.

Fig. 12.—Mitosis of endothelial cell lining large vein in submucosa.

Fig. 13.—Giant cell in submucosa.

Fig. 14.—Two plasma cells migrating through wall of vein in submucosa.

PLATE LVII.

Fig. 15.—Endothelium of vein shoved forward by a swollen plasma cell, two lymphoid and two large cells; the last are degenerating.

Fig. 16.—Another section of the same vessel showing fibrin forming around large, degenerated cells.

Fig. 17.—Vein occluded by a mass of fibrin.

Fig. 18.—Large phagocytic cells beneath endocardium of heart.

Fig. 19.—Mass of fibrin and degenerating cells beneath lining endothelium of a large vein in the submucosa. Mitosis of large cell at one end.

PLATE LVIII.

Fig. 20.—Mitosis of endothelial cell in capillary of liver.

Fig. 21.—Phagocytic cells in a portal vein in the liver.

Fig. 22.—Phagocytic cells occluding capillaries in the liver.

Fig. 23.—Mitosis of a large phagocytic cell containing a red blood globule in portal vein in liver.

Fig. 24.—A giant cell formed apparently in a capillary in the liver.

Fig. 25.—Phagocytic cells occluding capillaries of liver. Beginning necrosis of included liver cells.

PLATE LIX.

Fig. 26.—Focal area of phagocytic cells in capillaries of liver showing beginning degeneration with formation of fibrin.

Fig. 27.—Focal area in liver due to phagocytic cells in the capillaries. Necrosis of many of the included and adjoining liver cells.

PLATE LX.

Fig. 28.—Phagocytic cells undergoing necrosis in capillaries between trabeculæ of liver cells.

Fig. 29.—Large focal area in liver consisting of phagocytic cells. The included liver cells have entirely disappeared.

PLATE LXI.

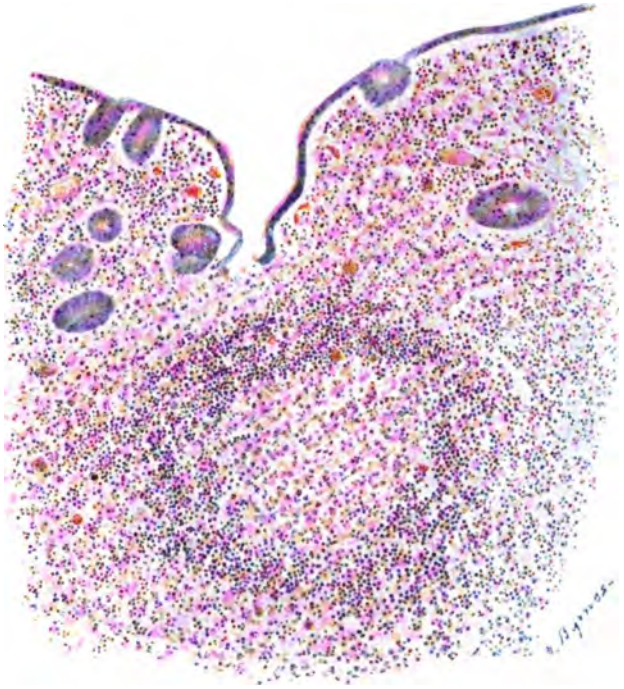
Fig. 30.—Large phagocytic cell in renal vein: judging from its inclusions it probably reached the general circulation through the thoracic duct.

Fig. 31.—Phagocytic cells in the exudation in the lung; from a case of lobar pneumonia complicating typhoid fever.

Fig. 32.—Group of cells in clot from left side of heart.

Fig. 33.—Phagocytic cells in a pulmonary blood-vessel.

Fig. 34.—Lining endothelium of large vein of spleen lifted up by phagocytic, lymphoid and plasma cells.



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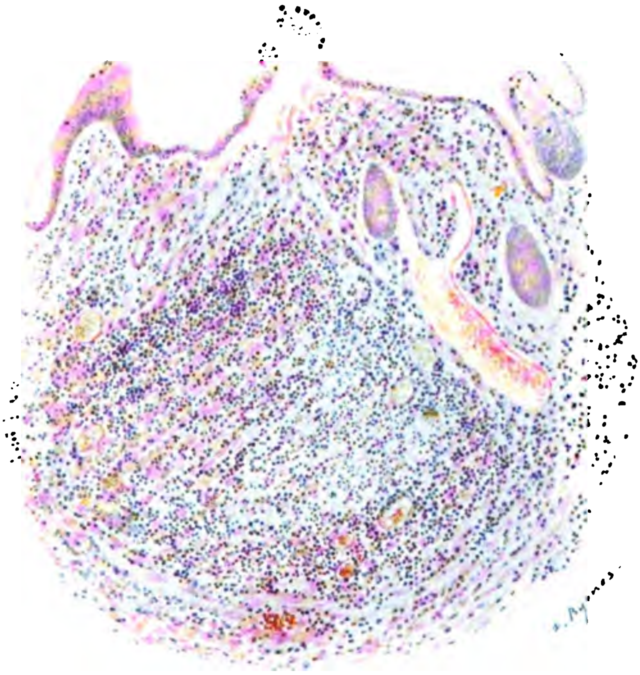

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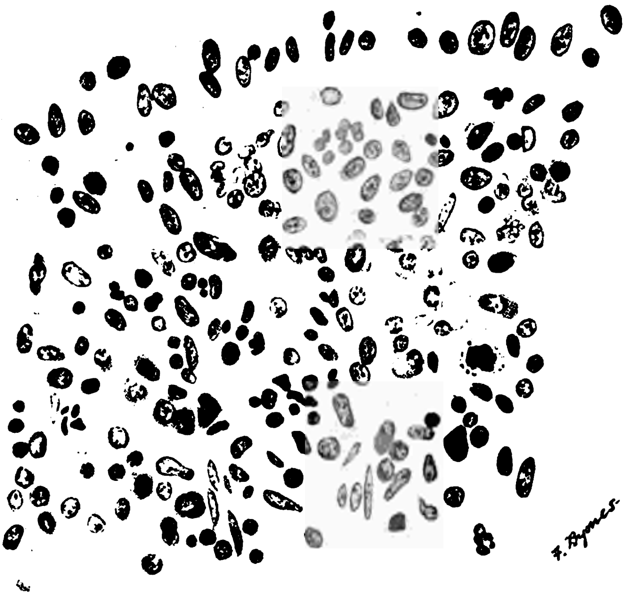

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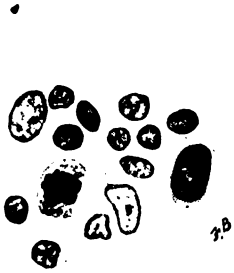

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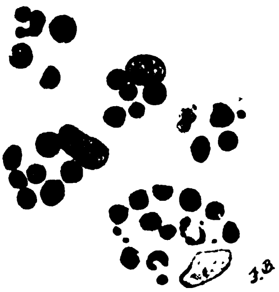

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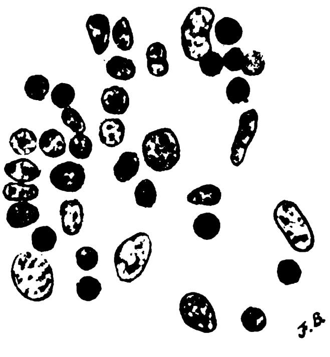

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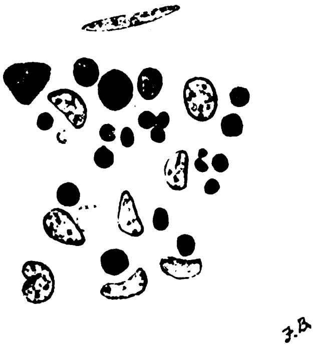

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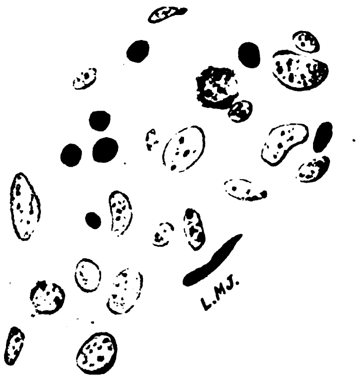

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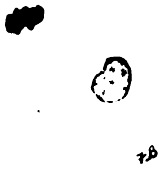

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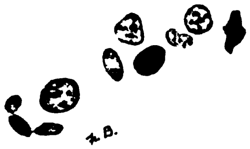

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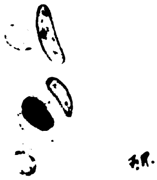

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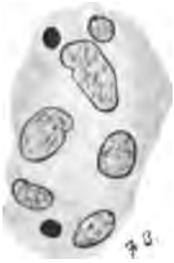

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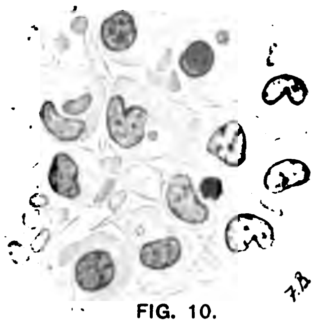

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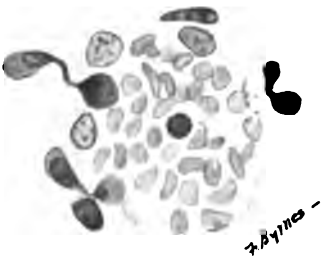

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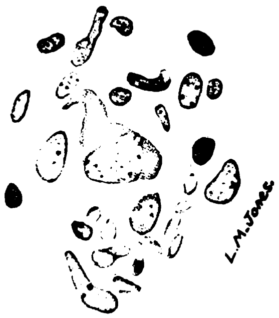

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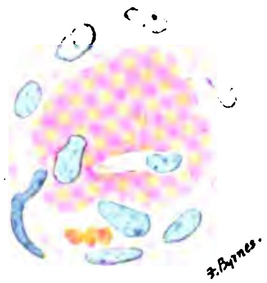

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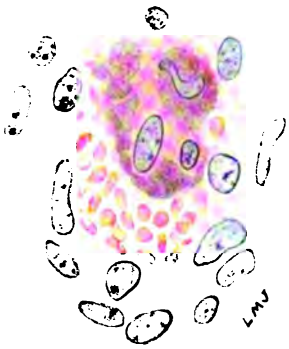

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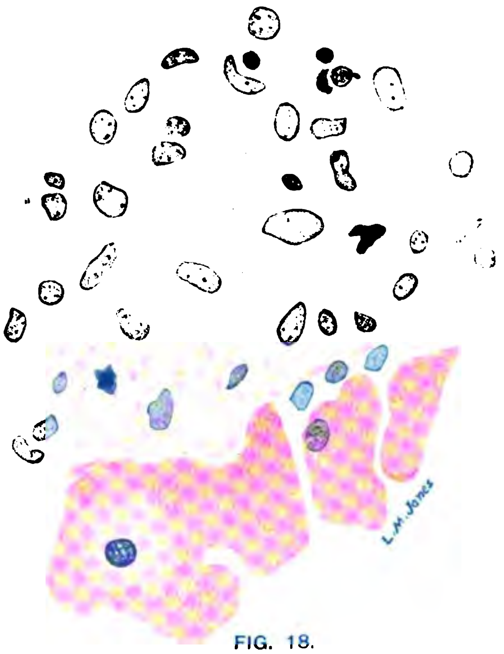

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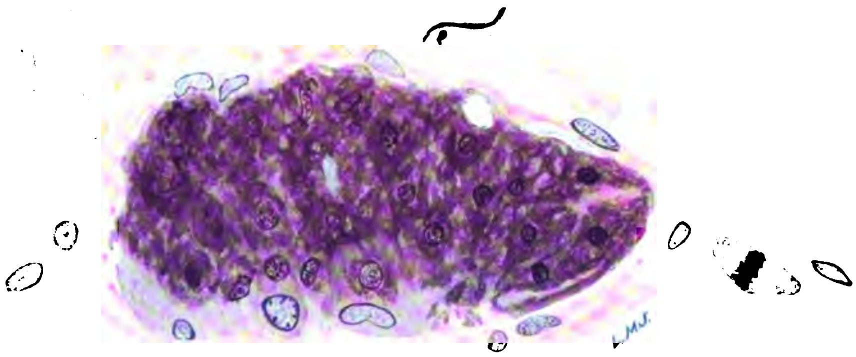

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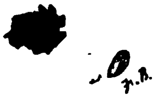

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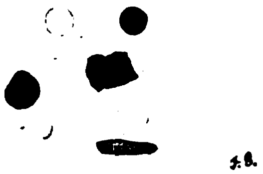

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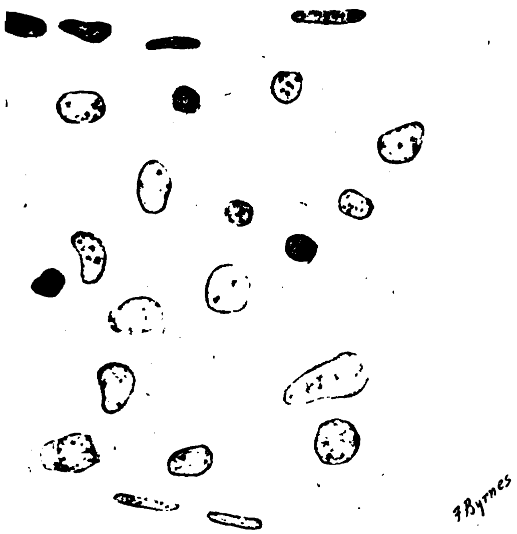

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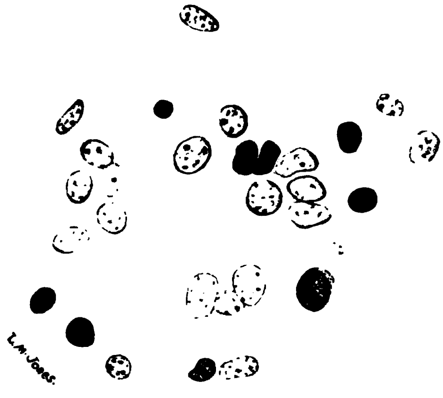

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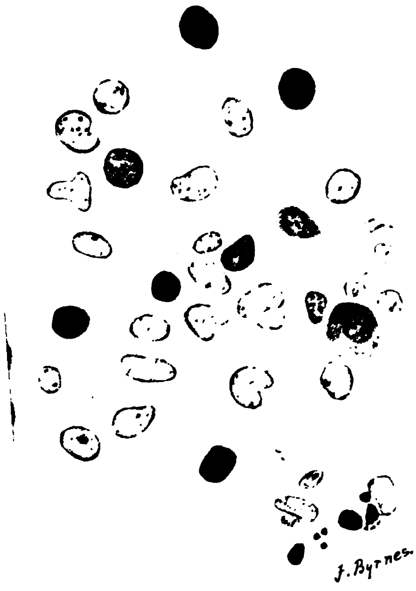

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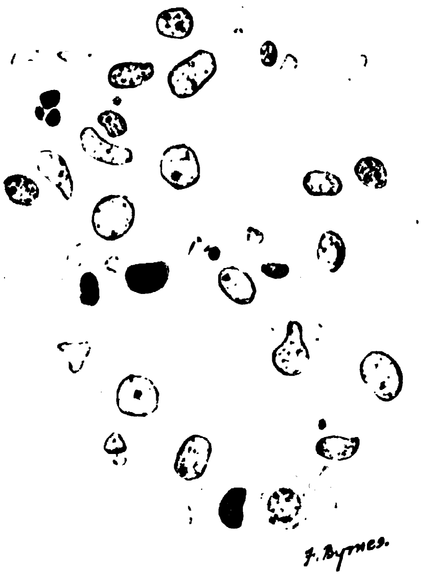

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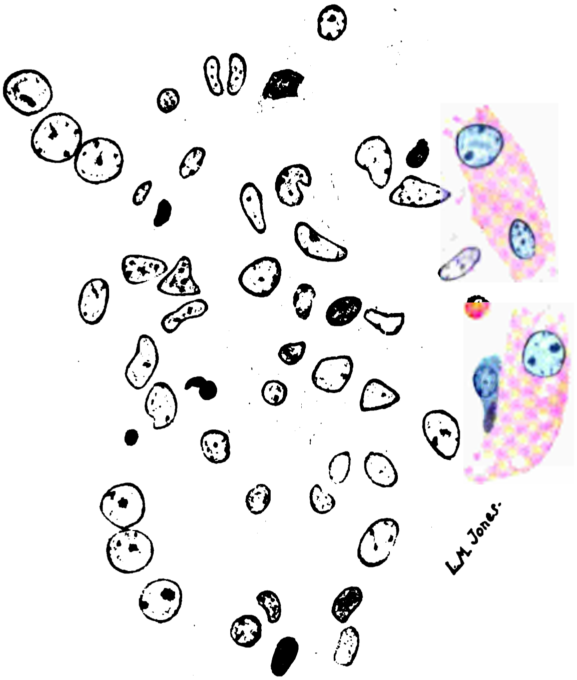

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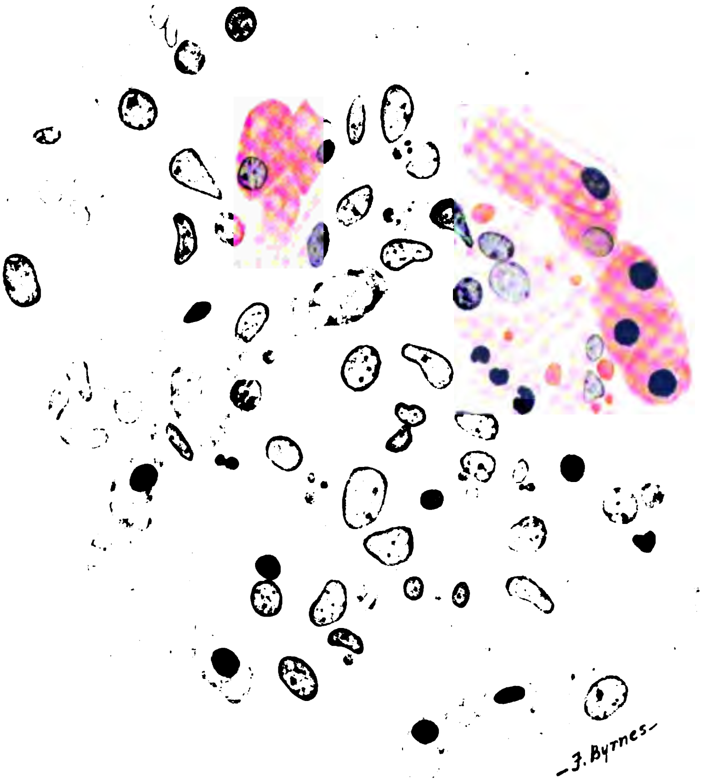

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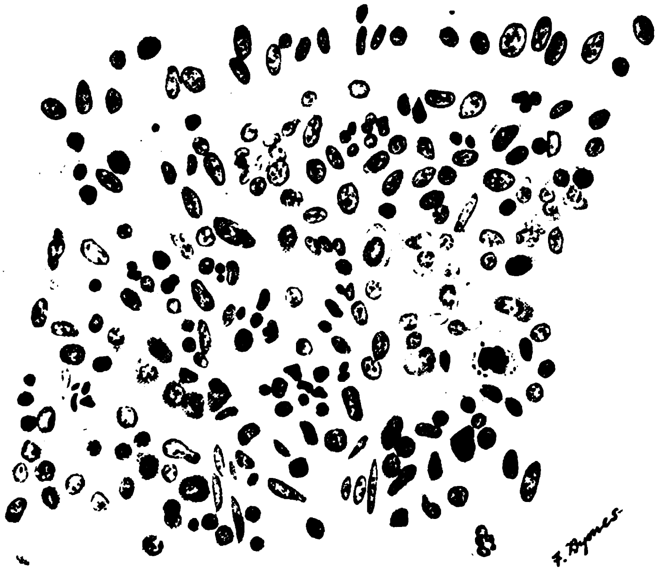

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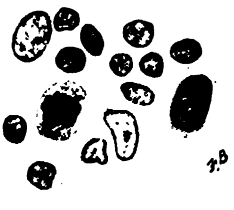

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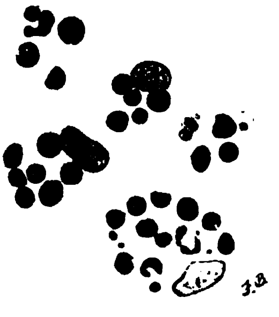

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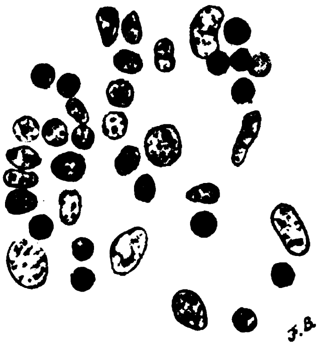

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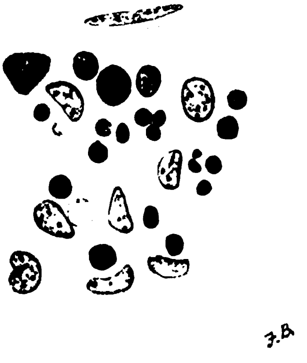

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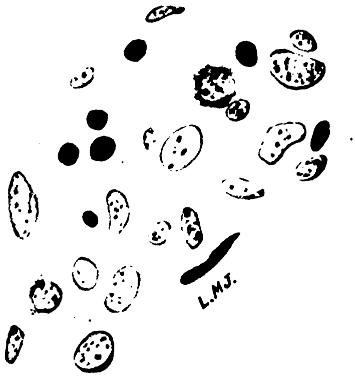

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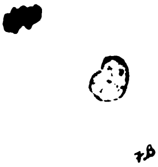

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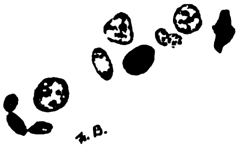

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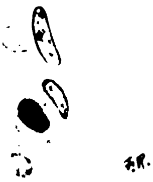

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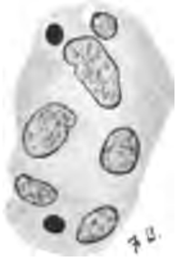

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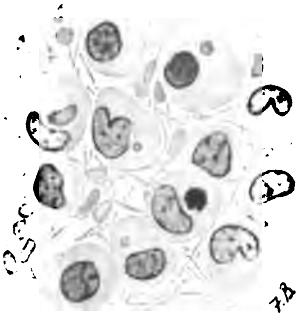

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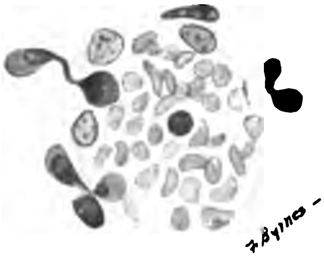

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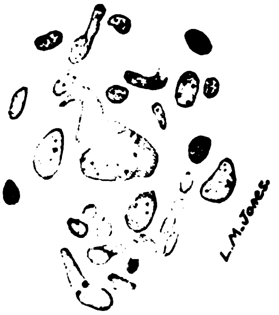

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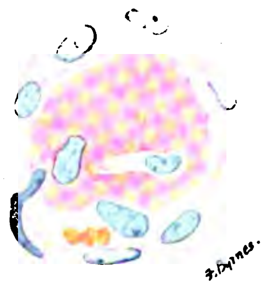

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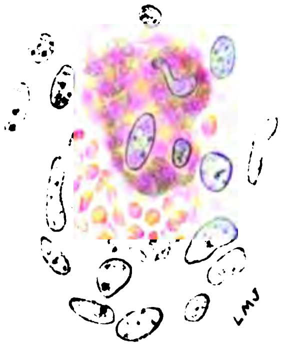

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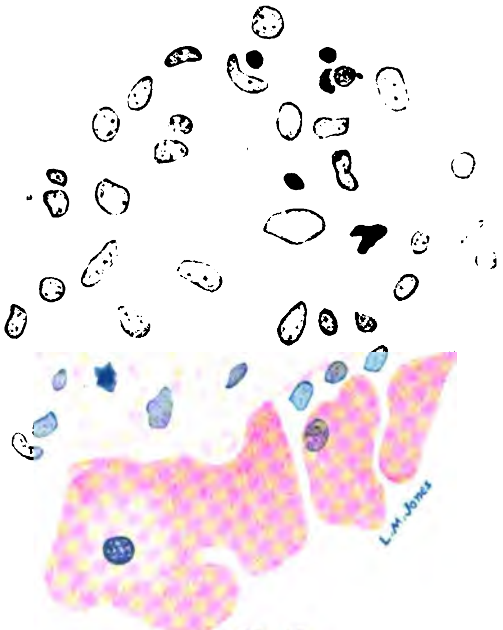

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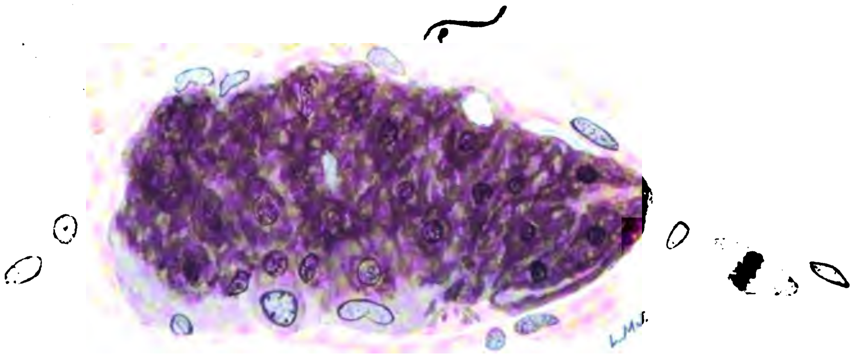

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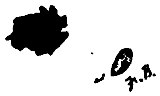

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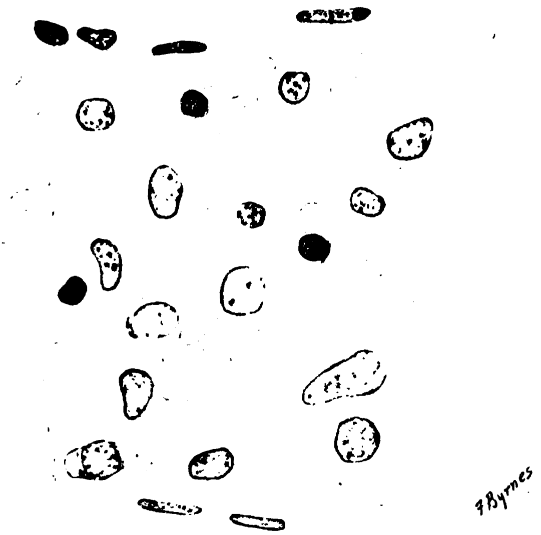

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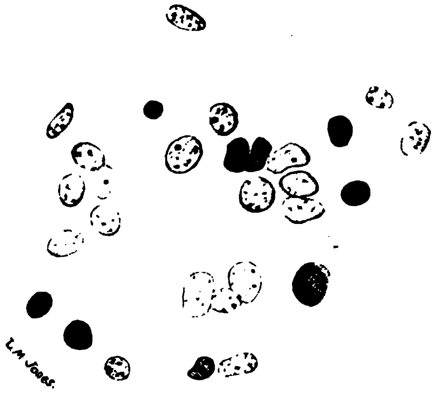

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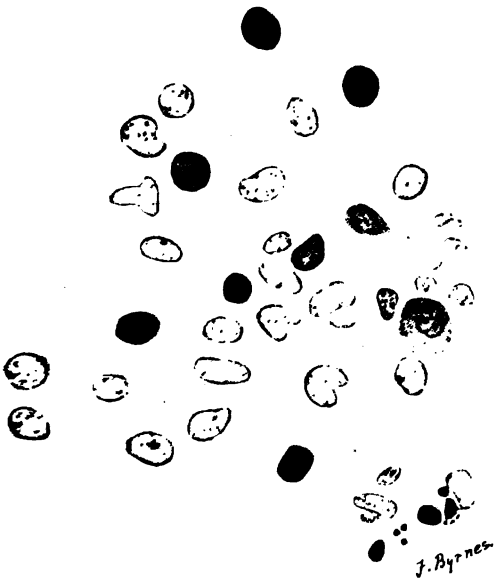

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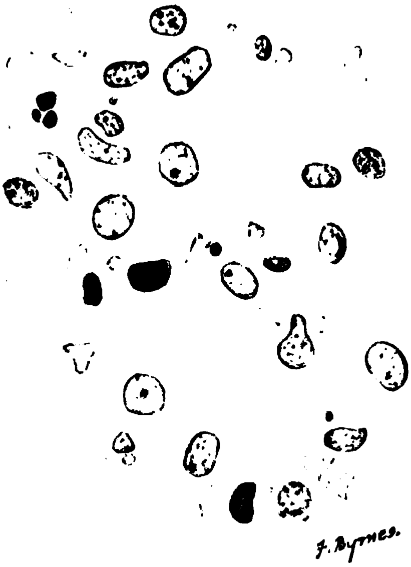

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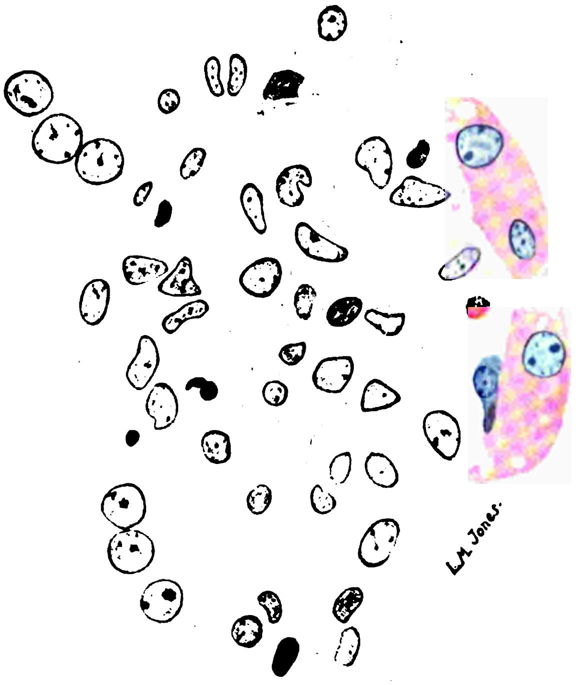

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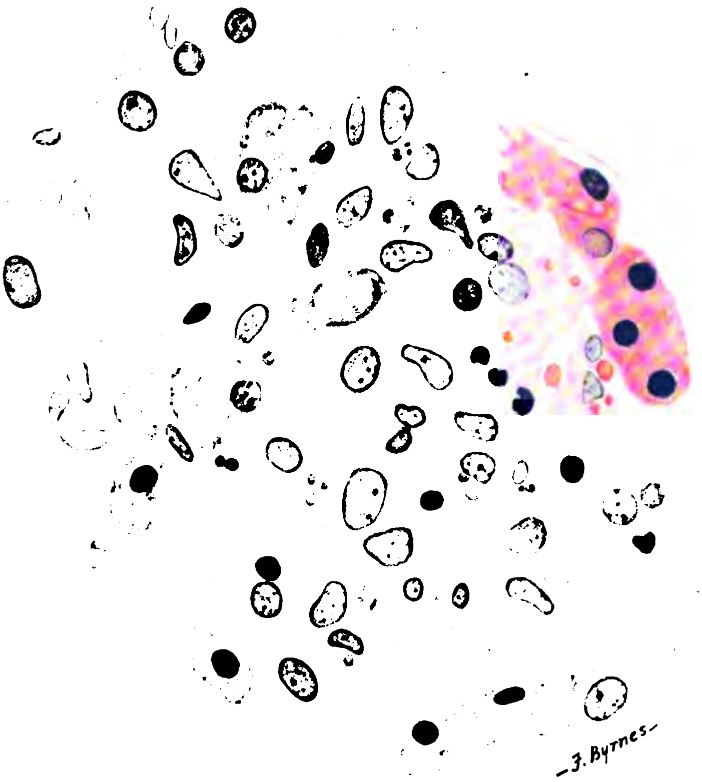

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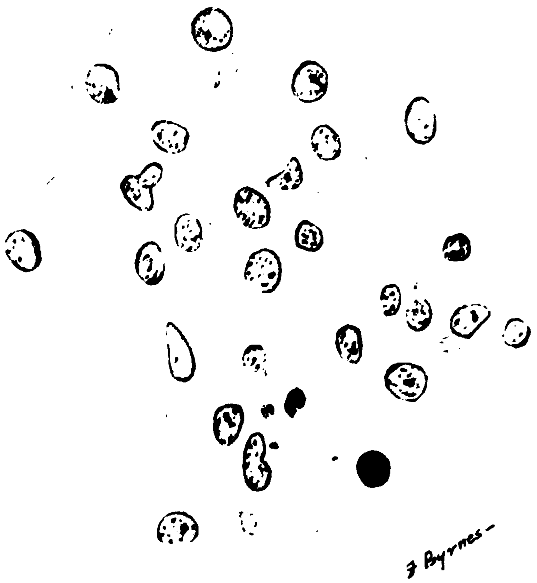

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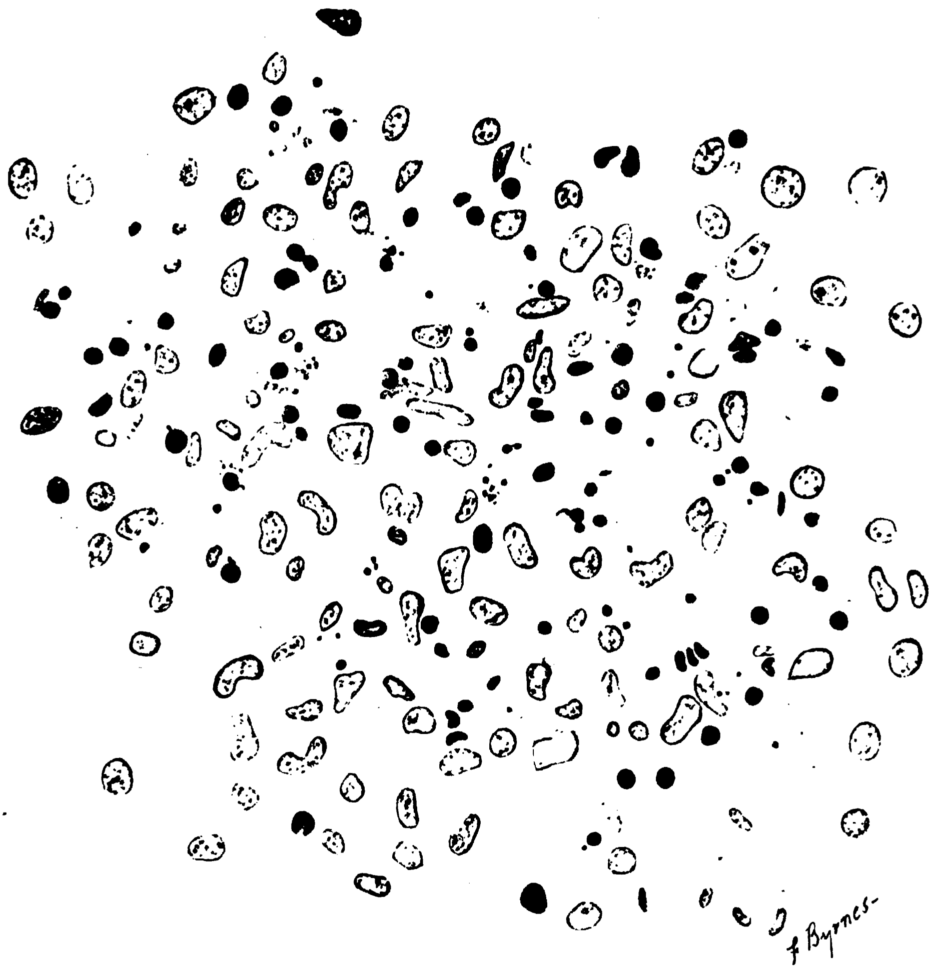

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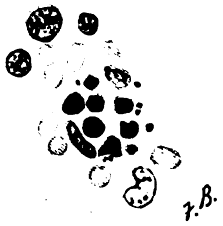

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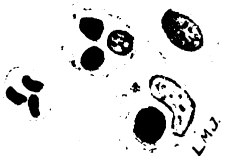

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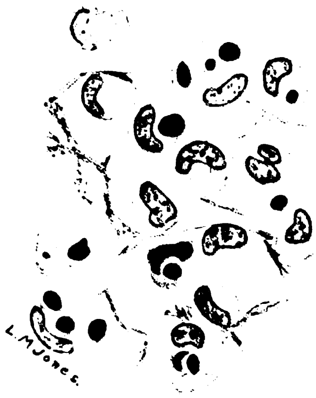

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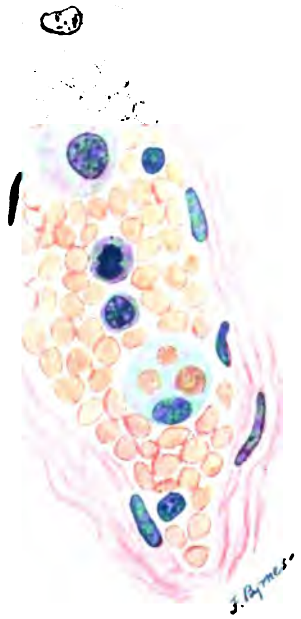

FIG. 33.

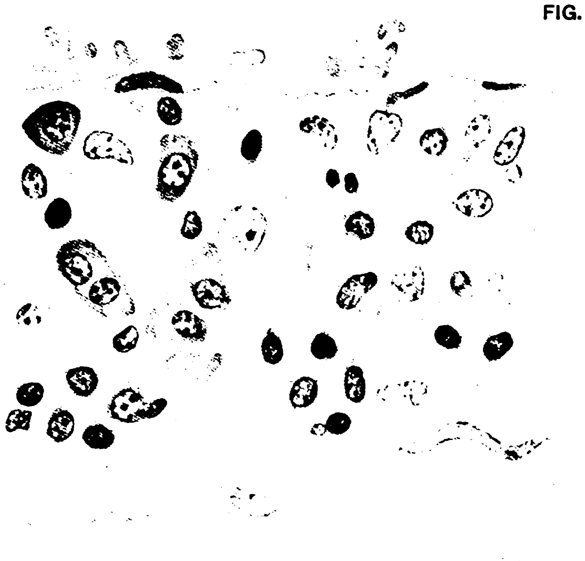

FIG. 34.

THE LEUCOCYTOSIS OF LABOR AND THE PUERPERIUM.

BY CLEON M. HIBBARD, A. M., M. D., AND FRANKLIN W. WHITE, S. B., M. D., OF BOSTON.

The first investigators to find a leucocytosis in pregnancy were Moleschott and Nasse (1). This observation has been confirmed by Mallassez (2), Halla (3), Fouassier (4), Virchow (5), Krüger (6), Kosina and Eckert (7), Limbeck (8), Rieder (9), and others. It has been found that most primiparæ have a leucocytosis in the later months of pregnancy, the average count being about 13,000. About 5 per cent of multiparæ have a leucocytosis, usually less high than the primiparæ. Virchow suggests as an explanation of the leucocytosis of pregnancy the gradual increase in size of the lymph vessels and glands about the uterus which occurs at this time, which then send a fresh supply of corpuscles into the blood. Rieder says there is no digestion leucocytosis in pregnancy.

A smaller number of observations have been made to determine the leucocytosis during and after labor. Kosina and Eckert found in 16 cases during labor 10,540 to 18,600 leucocytes per cub. mm. The count sometimes increased a little after delivery, but more often remained the same, and from the first day of convalescence gradually fell to normal. Krüger places the average in labor cases at about 13,000 leucocytes per cub. mm. Mallassez speaks of a rapid increase of leucocytes after delivery, followed by a gradual decrease to normal during convalescence. Rieder says the leucocytosis directly before and after birth is about two or three times the normal (7700) and falls rapidly from that time to the end of the puerperium. Cabot (10) counted 12 cases, 9 primiparæ and 3 multiparæ, during and after labor; the leucocytes varied from 10,000 to 37,000 in primiparæ, and from 11,000 to 16,000 in multiparæ. There was a rapid fall in count after confinement. Concerning the kind of white corpuscles causing the leucocytosis in pregnancy, Björkman (11) says, " In the last stages of pregnancy we find an increase of the white mononuclear elements very manifest and constant in primiparæ." Rieder gives differential counts of two cases, one multipara and one primipara, with a leucocytosis of about 13,000, and says this is not due to an increased number of polynuclear cells. The different kinds of cells in these cases preserved practically their normal relations.

In puerperal sepsis Limbeck found no leucocytosis present. Kosina and Eckert say that in puerperal fever the count rises in proportion to its severity, and after the fever is over again falls. Rieder, Grawitz (12), Kanthack (13), and others, find a leucocytosis present. Cabot says a leucocytosis is absent in severe and mild cases, and present in those of moderate severity.

Limbeck affirms that the effect of lactation on leucocytosis is not known. He suggests that the increased consumption of food in lactation may increase it.

In the past year Dr. W. L. Richardson, visiting physician to the Boston Lying-in Hospital, kindly gave us the opportunity of making the following observations during his service. The blood of 55 hospital patients was examined with reference to the number and kind of white corpuscles present. Our object was to determine the amount of leucocytosis present in labor and in normal convalescence after confinement, the peculiarities due to the age of the patient, and the beginning of lactation; also the effect of hæmorrhage, prolonged labor, septicæmia and inflammation of the breasts in causing departures from the normal. It was hoped that in doubtful cases of septicæmia and suppuration of the breasts the blood count might be a help to early diagnosis.

The blood was examined in the 24 hours preceding delivery, usually in the first stage of labor; also on the 1st, 3rd, 5th, 7th, 10th and 13th days after confinement (see Table I). Thirty-three of the patients were primiparæ, 22 multiparæ, the ages varying from 16 to 41 years. Taking 10,000 leucocytes per cub. mm. as the maximum normal, a leucocytosis was found to be present before delivery in 84 per cent of the primiparæ and 75 per cent of the multiparæ, the average for 32 primiparæ being 15,021, an increase of 50 per cent above the normal,

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Case No.	Pregnancy.	Age.	Stage of labor.	Time of count before delivery.	Duration of labor in hours.	Hæmorrhage.[1]	Temperature.[2]	Count before delivery.	Days of convalescence. 1st.	2nd.	3rd.	4th.	5th.	6th.	7th.
1	I	21	1st	5.20	11	Sl.	T.	9,600	14,380	15,000		10,100		14,800	
2	I	21	2nd	2.00	(?)	N.	T.	14,500	13,600		11,200		12,800	15,500	9,4
3	I	18	2nd	0.15	6	N.		14,400	11,700		7,000		9,400		9,
4	I	24	1st	1.30	14	N.	T.	15,000	8,600	19,060	19,300		12,800		7,2
5	I	23	1st	7.00	12	C.		16,800	17,200		11,730		9,200		10,0
6	I	23	1st	6.30	37	N.	T.	7,600	17,200		13,400		13,200		[illegible]
7	I	22	1st	2.30	8	Sl.		14,200	9,800		8,000		7,200		6,
8	I	22	1st	18.20	37	N.		11,400	10,500		11,600		8,200		6,1
9	I	23	2nd	0.30	6	Sl.		21,200	7,800		6,300		7,000		7,4
10	I	23	1st	7.00	20	Sl.		10,200	6,400		6,400		7,500		8,5
11	I	22			108	Sl.		13,000	8,800		6,500				12,5
12	I	20	1st	7.20	20	N.		15,600		6,900	8,200		7,500		
13	I	16	2nd	1.10	22	N.		21,000	13,500		8,100		12,000		
14	I	21	2nd	2.25	42	N.		30,600	19,400		10,400		10,100		
15	I	26	1st	5.00	53	C.	T.	14,100	18,600		9,600		13,600		10,
16	I	25	1st	4.00	17	N.		24,300	24,000			11,700			11,
17	I	17	2nd	0.15	25	N.		16,800	20,000			9,400			12,
18	I	18	1st	9.00	18	N.		8,100	9,800		11,600		9,600		9,2
19	I	17	1st	4.50	33	N.		18,400	15,600		9,400		8,800		9,5
20	I	22	2nd	2.00	12	N.	T.	12,600	10,600				8,600		
21	I	25	2nd	0.10	8	Sl.		21,900	15,300		11,900		8,000		[illegible]
22	I	21	1st	7.00	19	C.	T.	14,500	13,600	7,800	9,100		9,000		9,6
23	I	21			20	N.		17,000	8,400		13,600		5,600		7,
24	I	25			21	N.		19,400			8,300				12,0
25	I	17	1st	12.00	11	N.		9,800			10,800				14,0
26	I	23	1st	7.00	24	Sl.		11,200	7,800		10,000		7,300		8,4
27	I	17			3	N.		12,200	14,000		14,000		11,600		9,6
28	I	30	1st	27.00	41	N.		12,200	12,300		9,100		8,200		12,1
29	I	32	2nd	1.40	45	N.		15,500	12,000				7,600		10,0
30	I	27	1st	4.00	12	N.	T.	8,000	9,400		5,900		8,400		8,4
31	I	18			(?)	N.	T.	17,700	21,300						
32	I	17	1st	7.00	10	N.	T.	11,900	20,200		14,000		19,000		17,2
33	I	20			31	P.P.	T.		23,800			11,800		9,400	
34	II	22	2nd	0.25	7	Sl.		8,400	9,900		8,600		9,100		9,0
35	II	22	1st	1.00	8	C.		12,000	8,200		11,000		15,200		19,3
36	II	19	1st	5.15	30	Sl.		8,000	9,300		6,000		9,400		7,7
37	II	24			7	C.		10,100	10,600		7,600		6,100		6,0
38	II	26	1st	1.45	9	Sl.		10,200	9,600		7,200				11,1
39	II	30	2nd	0.50	6	P.P.		9,900	12,300		12,000		11,200		
40	II	23			12	N.		20,500		10,000			6,600		7,0
41	II	22	2nd	1.00	4	N.		14,200	6,000		12,800		10,400		12,3
42	II	32	1st	3.00	8	N.		12,500	12,100		12,700		9,600		11,
43	III	33	1st	2.00	12	Sl.		15,700	12,300		12,000		9,400		8,6
44	III	27	1st	1.00	7	N.		13,200	7,200		6,800		6,800		10,5
45	III	26	2nd	0.55	9	N.		13,300	12,400		8,400		8,400		10,4
46	III	23	1st	1.00	8	Sl.		11,100	12,300		7,600		10,000		10,0
47	III	30	2nd	1.30	33	C.		8,000	8,800		3,000		5,500		
48	III	25	1st	1.20	10	C.	T.	12,800	16,100		8,800				8,2
49	III	30	1st	3.00	(?)	N.	T.	10,200	11,200		10,100		7,000		
50	IV	39	1st	1.50	11	C.		17,200	10,800		8,000		5,900		10,6
51	IV	22	1st	1.30	4	N.		10,600	10,300		6,900		5,800		
52	VII	34	1st	5.00	10	N.		10,500	6,000		5,700		5,900		6,2
53	IX	33			8	N.		8,600	8,300		6,000		6,900		4,1
54	IX	35			6	C.			19,200		7,000		7,200		7,6
55	IX	41			11	N.	T.			18,000		8,700			

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[1] Hæmorrhage in delivery Sl. = slight, N. = normal, C. = considerable, P. P. = post-partum

T.

	9th.	10th.	11th.	12th.	13th.	REMARKS.
0			13,200			Macerated foetus.
0		7,600			2,900	15th, 10,900; 16th, 19,300; 17th, 10,000. Inflammation of breast on 5th and 16th days.
		10,600			8,000	
		12,500			15,000	Inflammation of breast on 8th to 10th day.
		8,000				
0		9,400			7,600	Sepsis.
		10,400			7,100	
		7,000			10,000	
		7,400			6,100	
		8,600				
		7,000			9,000	17th, 7,200.
		9,200			9,500	
		14,200			13,100	
		10,100			7,600	Inflammation of breast on 4th to 7th day.
		9,000				
		8,800				
		12,000			13,200	
		17,200			11,700	
		9,000			11,000	Temp. 101° on the 13th day, cause not known.
		10,600				14th, 9,400.
			7,400		5,000	Sepsis.
		6,100			9,400	
		9,200				
		11,700			11,500	
		10,600			8,400	
		10,300			6,900	
					8,200	
		11,200			6,200	
		7,800		10,300	6,000	Inflammation of breast on 12th to 15th day.
						Eclampsia.
		16,300		17,900		Anaemia, petit mal.
	10,300					Red corpuscles on 1st day, 1,500,000.
		7,400				
0		10,800			13,200	
		9,800				
		10,700				
		4,800				
	9,500				8,000	
		6,400				
		7,600				
		12,700				
		8,200			8,800	
		6,600				
		8,000				
		6,300	10,000		9,400	
		6,400			6,200	
		13,400				Alveolar inflammation on 10th day.
		5,500				Macerated foetus.
		16,800				
		8,300			6,800	
		6,700			5,500	
		9,400				Placenta praevia.
		6,600			9,800	Sepsis.

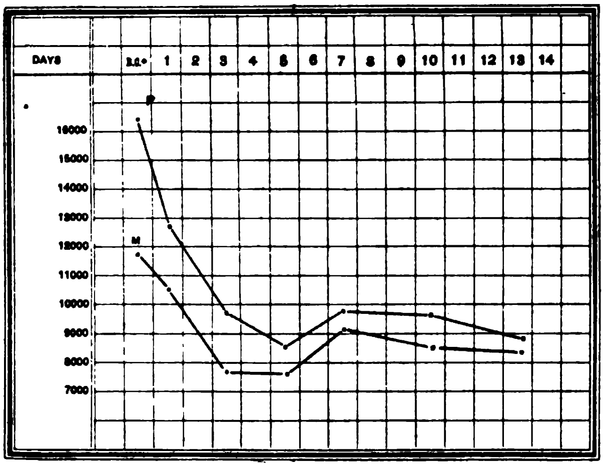
---

ge.

[2] Cases marked T. had a temperature over 100° during convalescence.

the average for 20 multiparæ being 11,700, an increase of 17 per cent. In 22 primiparæ and 17 multiparæ, the convalescence was perfectly normal and afebrile, and the average of the blood counts in these cases is taken to represent the normal leucocytosis of convalescence. The average for these primiparæ before delivery was 16,100, and for the multiparæ 11,800. The amount of the leucocytosis fell rapidly after delivery, reaching normal about the 4th or 5th day, then rose slightly till the 7th day, after which it gradually fell again to normal. This is graphically represented on the following chart (see Chart A). The curves for primiparæ and multiparæ show the same characteristics

CHART A.



*B. C =Before Confinement.

throughout, the number of leucocytes in the multiparæ being less. The disturbances in the breasts which commonly accompany the beginning of lactation, namely, swelling, induration and tenderness, are at their maximum from the 3rd to the 6th day after delivery, and it is interesting no note that the increase in the leucocytosis immediately follows this disturbance. We are inclined to regard this change in the blood as a result of the changes in the breast accompanying lactation. In considering the effect of age, the primiparæ were divided into two groups, those 21 years and under, and those over 21 years of age; and it was found that the leucocytosis in the younger women was above the average. The same was true of multiparæ divided into two equal groups, above and below 26 years of age.

The variation in the leucocytosis early and late in labor was next considered. The counts were made at different times before delivery, and on dividing the counts in primiparæ into two equal parts, according as they were made within four hours of delivery or earlier, it was found that the early counts averaged 12,200 and the late ones 17,600. The multiparæ showed a similar but less marked contrast between the early and the late counts, 9300 and 12,00 respectively.

Effect of Haemorrhage.—The average blood counts of 5 multiparæ who lost considerably more than the usual amount of blood after delivery showed a leucocytosis about 1500 above the normal average the day after confinement. Two cases of severe post-partum hæmorrhage (Nos. 33 and 39; see table), one a primipara, the other a multipara, showed a leucocytosis of 3000 or 4000 above the normal average for the first five days after delivery, the worst case having 1,500,000 red corpuscles and 23,000 white corpuscles the day after confinement.

Inflammation of the Breast.—Four primiparæ (Nos. 2, 4, 15 and 30) during convalescence had an inflammation of the breast, accompanied with fever of about 102°, congestion and tenderness, and in two cases with a small definite red indurated area in the breast. The febrile attacks were all mild, lasting only two or three days, terminating in complete recovery and without any indication of the presence of pus. In all these cases there was a marked increase of the leucocytosis just at the time of the febrile attack, which disappeared, however, immediately after it, the maximum counts being from 11,000 to 19,000. This is graphically shown on the accompanying chart (Chart B). Case 2 had two separate attacks of mastitis on the 6th and 7th days, and on the 16th and 17th days of convalescence. Case 15 had mastitis from the 4th to the 7th day. Case 30 from the 12th to the 14th day. Further observation of these cases is desirable, but it would seem that inasmuch as the blood count is so strongly affected by mild cases of mastitis, it can be of little help in early diagnosis of the severe purulent form.

Only three cases of *septicaemia*, all of moderate severity, came under

CHART B.

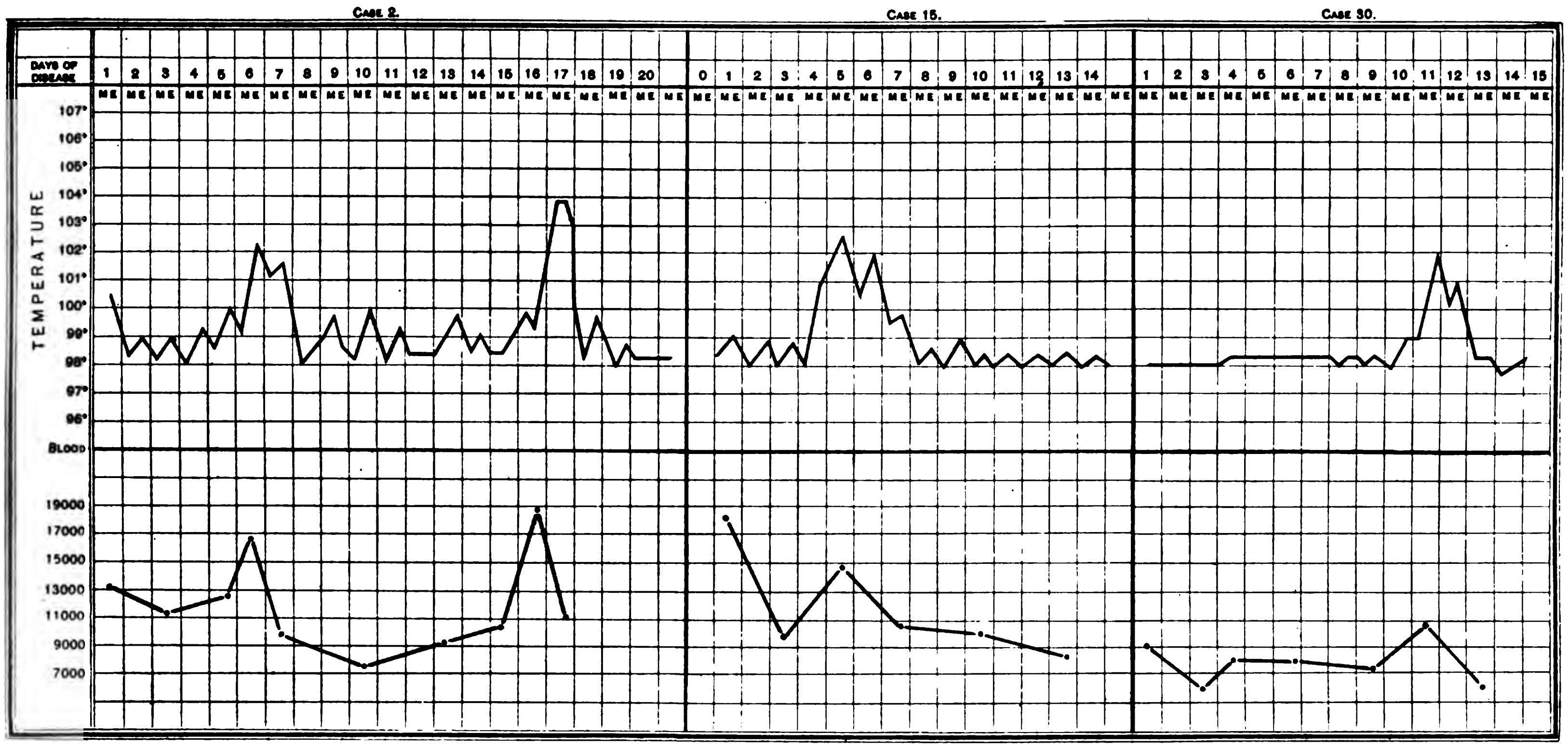
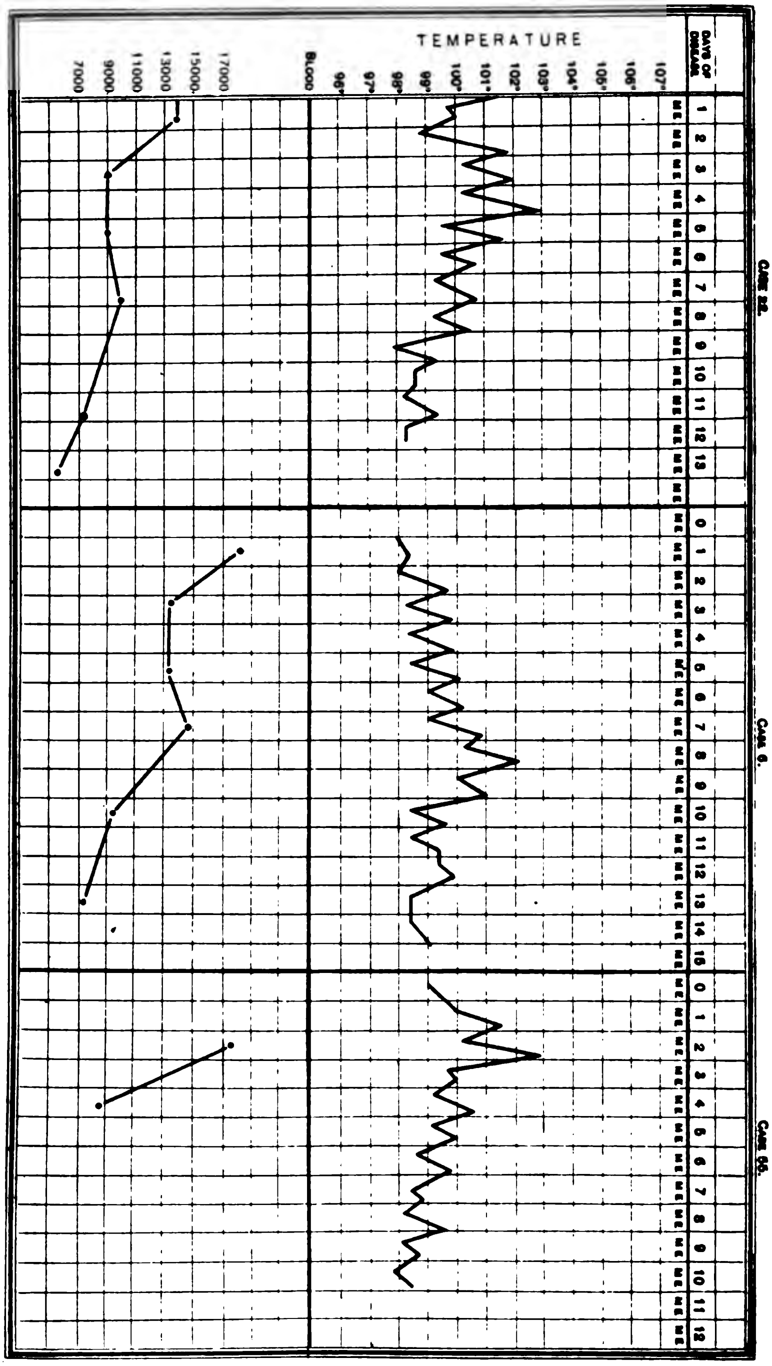

Case 2.
Case 15.
Case 30.
DAYS OF DISEASE
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
M E
TEMPERATURE
107°
106°
105°
104°
103°
102°
101°
100°
99°
98°
97°
96°
BLOOD
19000
17000
15000
13000
11000
9000
7000

CHART C.


TEMPERATURE
DAYS OF DISEASE
107°
106°
105°
104°
103°
102°
101°
100°
99°
98°
97°
96°
BLOOD
17000
15000
13000
11000
9000
7000
CASE 22.
CASE 6.
CASE 55.
1 2 3 4 5 6 7 8 9 10 11 12 13
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
0 1 2 3 4 5 6 7 8 9 10 11 12
M E

our observation. In Case 22 the leucocytosis ran a normal course; in Cases 6 and 55 there was a marked increase, the count varying from 14,000 to 17,000. When these patients had had the usual curetting and antiseptic treatment, and the fever and constitutional symptoms began to abate, the leucocytes subsided rapidly (see Chart C). Case 22 was curetted on the 3rd and 4th days; Case 6 on the 8th day; Case 55 on the 2nd day.

The septic cases are too few to serve as a basis for any conclusions.

It is interesting to note that in one fatal case of eclampsia (No. 31) the leucocytosis was 17,700 before delivery and 21,300 the following day.

TABLE II.

Case No.	Preg.	Leucocytosis.	Polynuclears.	Large Mononuclears.	Lymphocytes.	Eosinophiles.	Large Mononuclears. Absol. No. in 1 cmm.	Lymphocytes. Absolute No. in 1 cmm.
14	I	30600	93.2	1.8	4.4	0.6	552	1346
16	I	24300	90.	3.	6.	1.	729	1458
21	I	21900	95.8	1.2	3.	.0	263	657
9	I	21200	90.4	1.6	8.	0.8	339	1696
13	I	21000	95.2	2.6	2.	0.2	546	420
40	II	20500	91.	4.	4.	1.0	820	854
24	I	19400	94.2	4.4	4.4	.0	272	820
19	I	18400	86.2	2.6	10.	1.2	498	1840
5	I	17200	92.	3.	4.	1.	516	688
50	IV	17200	79.6	2.	16.8	2.6	344	2890
17	I	16800	89.	4.2	6.2	0.6	706	1042
4	I	15000	73.	5.	21.	1.	750	3150
2	I	14500	93.	1.6	5.4	0.	232	573
11	I	13000	80.	4.	15.	1.	520	1950
48	III	12800	86.5	4.5	8.1	0.7	576	1037
53	IX	8600	74.6	6.6	17.4	1.4	567	1496
34	II	8100	76.	6.4	17.2	0.4	518	1393
30	I	8000	74.2	7.2	17.9	0.7	576	1432
47	III	8000	72.	8.	20.	0.	640	1600

A differential count of the white corpuscles was made in 19 cases in labor, 12 with rather high counts, 4 with normal counts for comparison, and 3 of intermediate grade (see Table II). In the four normal cases the proportion of the different white corpuscles was practically normal. In the other 15 cases, with one exception (Case 4), the leucocytosis consisted in a marked relative and absolute increase of the polynuclear cells, the percentage of these cells being greater, as a rule, the higher the leucocytosis. There is a relative and slight absolute diminution in the number of lymphocytes, while the large mononuclear cells are relatively diminished and absolutely are normal.

Our results differ markedly from those of Rieder and Björkman. We will sum up our conclusions briefly as follows:

(1). A leucocytosis was present in over three-fourths of our cases in labor, being more frequent and higher in primiparæ.

(2). During convalescence the count falls rapidly at first, later more gradually, to normal. About the 7th day there is usually a slight rise.

(3). The leucocytosis is usually higher in the younger women, regardless of the number of the pregnancy.

(4). The patients farthest advanced in labor have the highest counts.

(5). Breast inflammation, even when mild, causes a prompt leucocytosis, hence the blood count has no value in the early diagnosis of breast abscess.

(6). The leucocytosis present at the time of labor is due to an increase of the polynuclear cells.

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ONE OF THE CONDITIONS UNDER WHICH DISCONTINUOUS STERILIZATION MAY BE INEFFECTIVE.

BY THEOBALD SMITH, M. D.

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Since the process of sterilization by heat forms the foundation for all bacteriological work, we might take it for granted that this subject had been pretty well exhausted. New phases, however, appear with new kinds of work, and the discontinuous sterilization of large quantities of bouillon has been so unsatisfactory in my hands that I deem it worth while to present some facts upon this well-worn subject. Without doubt experiences similar to those related below have visited other laboratories, but thus far the causes have not been discussed. Text-books are, as a rule, silent upon the subject. Discontinuous heating at 100° C. is regarded as co-ordinate and interchangeable with the use of a more elevated temperature, and the whole subject is dismissed very briefly. Among a large number of text-books examined Abbott's Principles of Bacteriology was the only one found in which some qualifying statement is made in regard to this matter: "It must be borne in mind that this method of sterilization is only applicable in those cases which present conditions favorable to the germination of the spores into mature vegetative cells. Dry substances or organic materials in which decomposition is far advanced, where the conditions of nutrition favorable to the germination of spores are not present, cannot be successfully sterilized by the intermittent method."*

The general proposition as here stated includes the subject I wish to discuss. The illustrations given do not, however, strictly apply to the bacteriological laboratory, since dry or decomposed substances are rarely dealt with there. A more apt illustration is the condition to be described.

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* Second edition, p. 53.

For more than a year past bouillon steamed in shallow layers (2 to 3 cm. deep) in the Arnold sterilizer for an hour on 3 or 4 successive days, though remaining indefinitely clear if unused, showed after some days' growth of diphtheria bacilli the presence of anaërobes. The source of the contamination was at first looked for in some error of manipulation during inoculation and in the stock culture employed. Soon, however, both these sources were excluded and the infection was referred to spores which had survived the boiling. It is interesting to note here that the bouillon became favorable to the germination of these spores and the multiplication of the bacilli only after the diphtheria bacilli had formed a membrane on the surface and were appropriating all the available oxygen. None of the flasks were ever found harboring aërobic spore-bearing bacilli.

Similar favoring conditions have been observed in the past on the surface of solid culture media when aërobes and anaërobes were introduced together. That the former may arouse slumbering spores of the latter lurking in culture media has not, I believe, been suggested, although the matter is self-evident. This experience is, as it were, an echo of the prolonged controversy which raged over the hypothesis of abiogenesis. Even at this day the acuteness of the intellects that finally solved the problem by satisfactory demonstrations is forcibly impressed upon the stumbling experimenter. In the case before us the conditions are somewhat different from those formulated by the participants in this controversy. We have a "putrescible" fluid which remains limpid. Inoculated with one bacillus, it apparently gives rise to another wholly different organism. The constancy of the second form in all the flasks examined, a large bacillus with a terminal spore, might in a more primitive state of our knowledge concerning bacteria have led to seemingly reasonable speculations concerning "the mutation of species" and the "spore-bearing phase of the diphtheria bacillus."

Discontinuous sterilization cannot therefore be relied upon when shallow layers of fluid freely exposed to the air are to be freed of bacteria. The spores of anaërobes accidentally present cannot find the conditions favorable to their germination in the intervals between the successive applications of heat. The autoclave is therefore indicated, for a temperature of 110° to 115° C. definitely rids the fluid of them. In short, a bacteriological laboratory would be seriously crippled without apparatus furnishing temperatures above the boiling point. But by a round-about method discontinuous sterilization may be even here made successful.

Having cultivated anaërobes for a number of years without the use of hydrogen in vessels having a restricted communication with the air, I filled round litre flasks with bouillon to the neck and had them steamed three of four times and then placed in the incubator. In several instances they became suddenly turbid after 2 days by reason of the almost explosive rapidity with which anaërobic bacilli multiplied, thus proving the theory of imperfect sterilization of the bouillon used for the cultivation of diphtheria bacilli. Most of the flasks remained sterile after 4 steamings with intervals of incubation between them gradually lengthened to 48 hours. It is not to be denied that there may be still other anaërobes whose sensitiveness to aërobic conditions is so great that development may not take place even in the nearly full round flasks. In several instances bouillon prepared for the cultivation of tetanus bacilli became clouded with anaërobes after four or five steamings, indicating that even under favorable conditions spores of this character germinate with difficulty, and that not less than 48 hours of incubation should decide the sterility of the fluid. It is thus possible to eliminate anaërobes without the autoclave. If the bouillon is to be used in shallow layers, it must finally be poured or siphoned under suitable protection from the round flasks in which the sterilization was effected into the sterile culture flasks, but this round-about process will rarely be needed, since it does not appear that 110° to 115° C. is any more harmful than 100° C. to the nutritive quality of the bouillon.

This subject is of some importance in other respects. It is possible that now and then anaërobes present in tubes of milk, for instance, may remain dormant until some other organism is introduced. Subsequent changes in the milk due to the anaërobe may be ascribed to the organism introduced unless the sediment be carefully scrutinized with the microscope. In tubes containing agar, gelatine, or other solid culture media, the restricted supply of oxygen in the bottom of the tubes will favor spore germination, so that the danger of subsequent contamination even when the autoclave is not resorted to is not likely to cause any trouble.

Sterilization from the point of view developed in these pages consists essentially in the destruction of all those bacteria or their spores which may subsequently find favorable conditions for development. The discovery by Globig* of very resistant spore forms and of bacteria which multiply only at a certain elevated temperature makes this definition accord with the facts. Inasmuch as our only test for the presence of life in any culture medium is its increase until visible mainly to the unaided eye, we are unable to affirm the non-existence of any life at all, although for practical purposes we may safely take it for granted after anaërobes have been destroyed.

The spores of adventitious anaërobes will probably be regarded as coming from contact of the beef with fecal matter at the abattoir—dust, dirt and utensils. There is, however, another source to which my attention has been called. In adding bits of tissue as large as peas from the organs of chloroformed rabbits and guinea-pigs, and in one case from the kidney of a pig slaughtered for food, to gelatine in tubes or to fermentation tubes pure cultures of anaërobes were obtained in four cases. It is thus not improbable that beef may contain a few stray spores which during the life of the animal have been carried from the digestive tube into different parts of the body, but which cannot germinate under prevailing conditions.

The various cultures thus far obtained from insufficiently sterilized bouillon and from animals have been reserved for further study and for possible identification with described forms. At present I may simply state that while they are all gas-producing bacilli bearing terminal spores, they evidently belong to different species or varieties of species, if we are to judge from the quite different odors and the variations in the gas formula $\frac{H}{CO_2}$ in dextrose bouillon as roughly determined in the fermentation tube.

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* *Zeitschrift f. Hygiene*, iii (1887), 294, 322.

NOTES UPON AN EPIDEMIC OF FOWL CHOLERA AND UPON THE COMPARATIVE PRODUCTION OF ACID BY ALLIED BACTERIA.

BY CHARLES H. HIGGINS, B. S., D. V. S.

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The bacillus of fowl cholera, although previously observed by Perroncito and others, was first successfully cultivated in 1880 by Pasteur, whose investigations of this organism mark an epoch in the bacteriological study of disease. Although this bacillus is well known and has been fully described, it has not been positively identified as the causal factor of disease upon this continent. Cholera in poultry, it is true, exists here, and in Bulletin No. 8 of the Bureau of Animal Industry there is to be found a very careful study of "Three Outbreaks of Fowl Cholera" by Dr. V. A. Moore. These epidemics occurred in places far apart, one near Providence, R. I., another in Virginia, and another near Washington. The germ from all three proved to be the same. It differed, however, in its characters from the European bacillus of fowl cholera, though closely allied thereto. The germ that I am about to describe would seem closely to resemble, if not to be identical with, the true bacillus of fowl cholera as found in various European countries.

The fact, then, that I have been able to isolate a germ which, while closely related to that causing outbreaks of a very similar nature along the Atlantic coast, has been found after prolonged study to be distinct from this form, would seem to be sufficient ground for publishing a description of the organism and its characters. In studying this form I have been enabled to pursue a series of investigations upon certain methods for the differentiation of species. Although I cannot state that these studies have led to complete success, it will, I think, be well to mention those employed by me, so that others may know how much has been done along these lines and with how much success, and may possibly carry on the work to a more successful conclusion.

I have more especially attempted to arrive at methods for the study of the comparative acid production by allied forms of bacteria, employing germs of various virulence from this particular outbreak, together with forms isolated from the epidemics of fowl cholera in the States and cultures of the bacillus of the disease received from Europe. To gain a sure basis for such investigations I have endeavored to manufacture synthesized media so that I might more surely start from known compounds capable of being split up by bacterial growth. The number of synthesized media that I have produced* has been very great, but my success in the matter has only been partial, for the bacillus of fowl cholera and its allies grow but poorly upon nearly all the fluids made up by me. Nevertheless, I feel that even my partial success has given me fuller knowledge of this subject of acid production by bacteria, and the results obtained may possibly be deemed of value as throwing some light upon at least one more possible method of differentiating between closely allied forms.

My attention was called to this outbreak December 5, 1895, by Professor Adami, who had in turn heard of it through Dr. G. P. Girdwood. The outbreak occurred among a flock of fowls belonging to Mr. A., of St. Anne's, P. Q. St. Anne's is situated about 20 miles from the city of Montreal, on the island of Montreal, where the St. Lawrence divides to encircle the island. On the 8th I visited Mr. A., among whose fowls the outbreak had occurred.

History.—The first signs of the disease had been noticed about a month previously, the animals dying very suddenly. The disastrous results were communicated to Mrs. G., among whose fowls an outbreak had occurred several years previously. She suggested the placing of sulphuric acid and sulphate of iron in the drinking water and allowing access to no other water, also a complete disinfection of the hen-house. After cleansing the coop and administering the above remedy the disastrous results were rapidly checked, but few fowls having died since. The disease pursued a very rapid course, producing death in from 3 to 12 hours from the time that the first signs were noticed. The greater number were found dead in the morning (from two to four at a time). In all there were lost about 20 fowls, which were invariably among the youngest. The older ones seemed immune and none showed symptoms of the disease.

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* See addendum to this paper.

Symptoms.—From the outset there was a profuse diarrhœa, yellow in color; the feathers were ruffled and there was a yellowness about the head. The fowls were not inclined to move after noticeable symptoms occurred, but rather showed a tendency to roll themselves up in a ball, so to speak, and to remain motionless till they fell over dead.

The hygienic conditions were favorable for the development of any infectious disease. The fowls were confined in a coop about ten feet square and six feet high. There were two small windows, one on the east and the other on the west elevation, each being about two feet square. At the time of my visit the place was damp and was said to have been particularly so at the time of the outbreak. After the outbreak a fire was started in order to dry out the apartment. There was little or no ventilation.

Three animals dead of the disease in question were procured. The exact length of time they had been dead was not definitely known, but it was thought to have been at least a week. Upon the death of the fowls they were immediately thrown outside and were at the time of the visit covered with snow. For several days it had been very cold; there was a snow-storm on December 2nd and another the night before the visit (that of December 7th). To all appearances the fowls had been covered by both falls of snow. Probably they had been exposed during the time they had lain outside to a mean temperature of at least — 20° C., it having been very cold after the first fall of snow. On the night of the 8th they were left in a sleigh out of doors, a thermometer nearby registering —16° F. or —27° C. at 8 A. M. on December 9th. They were brought to the Molson Pathological Laboratory of McGill University on the morning of the 9th.

Morbid Anatomy.—The appearances found at the necropsy will be cited for but one case, as there was little or no difference noted in the three. The subject was a mongrel Plymouth Rock hen about eight months old. She was in good condition. The liver was rather pale with areas of necrosis. Spleen soft, pulpy and enlarged. The intestines were pale externally and free from evidence of hæmorrhage. Within, the mucosa was congested and swollen. The lower portion of the intestines was particularly affected, and its follicles were moderately distinct. There was fluid in the pericardial cavity, with some hæmorrhage beneath the pericardium, and marked bloody imbibition of the vessel walls of various organs.

Cultures were made from various organs and fluids of the body, and revealed on the following day a pure and moderately vigorous growth of a non-motile bacillus. The bacillus in all the cultures from the same fowl appeared to be identical; the cultures from the various fowls were found to be identical and pure.

DESCRIPTION OF THE BACILLUS.

Morphology.—The micro-organism is a non-motile, rod-shaped organism, aerobic, though growing in the presence of little free oxygen, varying slightly in size according to the medium upon which it is grown. In broth at 37° C., at the end of 20 hours, the bacilli vary from 1.3 to 1.8 μ in length and from 0.5 to 0.7 μ in breadth. The most frequent form is a diplobacillus with rounded ends, which may under low magnification be mistaken for a diplococcus. In the hanging-drop preparation it shows a distinct polar arrangement of the protoplasm. It is frequently seen in old cultures in short chains consisting of three or four of the bacilli placed end to end. It stains with the ordinary aniline dyes but retains the coloring matter feebly or not at all when subjected to Gram's method. It appears in great numbers in the tissues and also in the blood. In the tissues it often occurs in clumps of four or five. No spores or any tendency to spore-formation have been recognized. A feebly-staining capsule has been occasionally observed.

Cultures in Broth.—This medium was made by using 2 grammes of Liebig's extract to 500 cc. of distilled water, 1 per cent Witte's peptone and 0.5 per cent rock salt. Kept at 37° C. the broth is uniformly clouded at the end of 24 hours. This cloudiness does not as a rule settle, but in some cases the broth eventually cleared, the conditions being kept unaltered. The germ was found to be still alive in tubes which had stayed in the laboratory for 9 weeks.

When ordinary broth is neutralized with hydrochloric acid or sodium hydrate, as the case may be, phenolphthalein being used as an indicator, the germ will grow between the limits of 4 per cent of a decinormal solution of hydrochloric acid and 4 per cent of a decinormal solution of sodium hydrate. Between these two limits the culture shows very little difference upon removal from the incubator at the end of 24 hours. Beyond these two points, on both the acid and alkaline sides, the growth is very feeble, and on tubes containing 8 per cent of decinormal hydrochloric acid and of those containing 10 per cent of decinormal sodium hydrate solution there is no growth at the end of 24 hours. It may be added that there is a decided odor characteristic of broth cultures of this germ.

Fermentation (gas production) experiments by means of the Smith tubes proved negative with both 1 per cent lactose and 1 per cent glucose broth.

Nutrient Gelatine Cultures.—Here the Liebig's extract broth was used, 7 per cent of gelatine being added. This when made up was slightly acid (to phenolphthalein), but the acidity was well within the limits determined by the previous experiments with broth cultures.

The growth on the nutrient gelatine is less vigorous than in broth and is recognizable in stab cultures and on plates about the third or fourth day. In stab cultures there is little or no growth at the surface, the growth being equal all along the line of inoculation. On plates, superficial colonies about 0.5 mm. in diameter are seen on the third or fourth day; the deep colonies are more minute. The surface growths are granular without sharply-defined borders. The deep colonies show a granular appearance; their color is a very pale white. There is no liquefaction. It was noticeable that no cultures were obtained beneath mica plates. In other words the bacillus will not grow when deprived of free oxygen, but grows better where there is little oxygen (along the stab in stab cultures) than where oxygen is abundant (on the surface).

Agar Cultures.—The Liebig's extract broth was used for this purpose, 1 per cent of agar being added. This also was slightly acid to phenolphthalein, but the acidity was well within the limits of active growth. Growth on this medium at 37° C. is not very vigorous; at the end of 24 hours there appears a very pale white, almost colorless streak along the line of inoculation which has a glistening appearance. Isolated colonies are about 0.2 to 0.5 mm. in diameter, convex with granular edges. There is acid production, but this is not abundant. Lactose litmus-agar shows a red coloration along the streak on the second day. Only after about 4 days is there a reddening of the surrounding medium, and even then this is not very extensive. A peculiar penetrating odor arises from both tubes and plates.

Potato Cultures.—Upon this medium the growth is not very vigorous, but is present as a slight yellowish coloration along the line of inoculation after 4 days.

Milk.—A change is noted in the consistency of the cream after 4 days. Other than this no change is to be recognized in milk cultures kept in the incubator for 6 weeks.

Blood Serum.—Natural blood serum coagulated was used. The growth is very similar to that obtained upon agar, but is not nearly so vigorous. The growth along the line of inoculation is seen after a few days to broaden slowly if the tube be kept in the incubator.

Temperature Relationships.—The germ grows at ordinary temperature and also at that of the blood. It resists a heat of 59° C. for 10 minutes; it will not withstand one of 60° C. for that length of time. These relationships were determined according to the instructions laid down in Sternberg's Text-book of Bacteriology, 1896. As already pointed out by me the germ is evidently capable of resisting a temperature below 0° C. for several days and of —27° C. for several hours, although evidently this extreme cold led to some attenuation.

Action of Disinfectants.—A 1 per cent solution of carbolic acid destroyed the life of the germ in 5 minutes. A half per cent solution of hydrochloric acid was fatal in the same time, as was also a half per cent solution of sulphuric acid. The commercial hydrochloric and sulphuric acids were employed in these experiments.

RESULTS OF INOCULATION.

Poultry.—A fowl inoculated intravenously with 2 cc. of a culture 24 hours old was found dead on the second day. Further than this the virulence for fowls has not been determined, on account of an accident, owing to which the virulent germ was lost. A few hours after inoculation the fowls became listless. Even with the most attenuated virus a few hours after inoculation a profuse diarrhœa was noticed, the stools consisting of semi-mucoid masses of a greenish-yellow or white color. The autopsy showed as follows: Intestine filled with gas, crop empty, gizzard filled with small stones and ingesta. Intestine contained a greenish-yellow, semi-mucoid mass, kidneys normal, spleen congested and enlarged. Kidney fatty. Gall-bladder much distended. Heart and lungs normal. Where the fowls had been given a subcutaneous injection of a less virulent form into the breast muscle, at the point of injection was a mass of cartilaginous hardness, of a yellow color, free in the muscle tissue. The bacilli were found in all the fluids and tissues of the body.

Immunity has been produced in young chickens by the injection of a moderate quantity of an attenuated germ.

Turkey.—A turkey was inoculated intravenously with 6 cc. of a 24-hours-old culture and showed symptoms about an hour afterwards. At this time he was loath to stand; the feathers were slightly ruffled and the head drawn close to the body. The symptoms further than this were not noted, as the animal was inoculated at night and found dead in the same spot on the following morning, having succumbed in about 15 hours. The autopsy, made a few hours after death, revealed the following: Liver, great congestion, otherwise normal. Heart, lungs, spleen and kidneys normal. Intestine, pale externally. From within, the mucosa was seen to be swollen and greatly congested, showing ecchymoses and areas of necrosis. This congestion was not localized, but extended from the gizzard to the anus. The intestine was filled with partially digested food of a greenish color. Crop greatly distended with ingesta. The mucous surfaces, from the mouth to the gizzard, were studded with ecchymoses and showed congested areas. This was also true of the mucous surfaces of the cavities of the head. The mucosa of the gizzard also showed congested areas.

*Rabbits.**—The first rabbit was inoculated through the digestive tract, and death occurred on the twelfth day. The disease followed the course of a septicæmia, the symptoms of which were characteristic. Another animal inoculated with a culture, 24 hours old, from this first died two days later. After this the germ rapidly gained virulence, the last rabbit, which was the ninth of the series, dying 6 hours from the time of inoculation. This last rabbit was inoculated with 3 cc. of a preparation of ten drops of blood from the previous animal to 10 cc. of broth. The lesions presented at the autopsy were as follows: Upon opening the abdominal cavity there was noted acute peritonitis with a slight effusion of serum. The intestine was distended with gas. The mucosa of the stomach peeled off with ease, the surface being studded with small ecchymoses and congested. The intestine was filled with a greenish-yellow, semi-mucoid mass. The mucosa was injected. Appendix discolored, mucosa congested with small ecchymoses. The large intestine contained gas and a dark semi-fluid mass. Liver firm, gall-bladder greatly distended. Kidneys slightly congested. Heart and lungs normal. The mucosa of the uterus showed great congestion and partial necrosis. The bacillus was found in the blood, and cultures from the various organs also revealed its presence. In this case it was demonstrated in the vitreous humor of the eye.

The symptoms of the inoculation disease in rabbits were marked. There was drowsiness. The hind legs were drawn well under the body.

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* Evidently the conditions under which the bacilli were gained, namely, from the bodies of animals which had been dead for several days, and had been preserved at a temperature far below 0° Centigrade, had induced a very definite attenuation of the microbes.

The forelegs were drawn as far back as possible. In the disease produced by the virulent germ there was profuse diarrhœa beginning a few hours after inoculation, and in those withstanding for some time small tubercle-like masses were found in the appendix. Rabbits inoculated with the most virulent virus developed symptoms of an acute disease, remaining motionless until death supervened. In the disease produced by the attenuated germ the symptoms were similar, but the animal took food up to about 24 hours before death, at which time acute symptoms developed.

A guinea-pig inoculated with 1 cc. inside the thigh developed a local abscess.

Dogs fed on dead subjects developed no marked symptoms save a profuse diarrhœa. There was no elevation of the temperature.

The bacillus here described corresponds closely in all respects with the bacillus of European chicken cholera. So close is the resemblance that I cannot but hold that the two are identical, and that I have been dealing with an epidemic of the true disease, the first recorded in America. In only two respects have I found slight divergences from the statements of previous observers, namely, in the resistance of the organism to heat and disinfectants. The European germ is said by Baumgarten to be destroyed by an exposure to 55° C. for fifteen minutes or, according to Salmon, by an exposure to 56° C. for ten minutes. The form here described was killed only by a temperature of 59° C. with ten minutes' exposure. On the other hand it would appear to be more sensitive to disinfectants, for whereas Hueppe found that the European germ was killed by 3 per cent carbolic acid in six hours, the Canadian form did not grow after being left in 1 per cent carbolic acid for five minutes. The discrepancy in both cases would appear considerable, but it must be remembered that the want of agreement in the results of various observers in connection with these two tests of thermal death-point and disinfectant action are notorious, so that, when these two tests alone yield divergences, we do not possess sufficient grounds for stating that we are dealing with separate species.

From the form already recognized in America the separation is clearly marked. The Canadian germ is smaller, takes the polar stain well, does not saponify milk, produces acid in dextrose and lactose broths, can be rendered most virulent for rabbits so that a drop or two of the blood of an affected rabbit will lead to the death of another in six hours, while both in fowls and rabbits there is developed a most profound diarrhœa. In addition to these differences I have found, in comparing the growths, that the American form flourishes much more vigorously in gelatine (stab and streak cultures), so that the growth is already recognizable in twenty-four hours, while the Canadian and European forms show nothing until the fourth day. On the other hand in plate cultures the colonies of the American form are much smaller, with more sharply-defined borders. There is little or no difference upon agar.

The study of the whole of the large group of bacilli of hæmorrhagic septicæmias has revealed so much variability, so many individual points of difference between microbes in the main closely resembling each other, that I shall not venture to say that the forms isolated by Dr. Moore belong to a distinct species. I only urge that the Canadian form approaches much more nearly to the classical type.

Before closing this section of my paper attention may be called to what, I believe, has not previously been noted, namely, that the turkey is very susceptible to inoculation chicken cholera.

UPON THE COMPARATIVE PRODUCTION OF ACID BY ALLIED BACTERIA AS A MEANS OF SPECIES DIFFERENTIATION.

In studying these three allied forms of bacteria I was led, thanks to Professor Adami, to pass beyond the limits of my initial research. His suggestion was that the comparative acid and alkali production by various species of bacteria had been insufficiently studied and that possibly a careful examination of such acid and alkali production might reveal a further means of differentiating allied forms. Acting upon this idea I have spent several months endeavoring to arrive at satisfactory conclusions with regard to the subject. Even now I cannot consider that more than preliminary studies have been made, nor should I publish at the present time were it not probable that an indefinite period may elapse before I can again take up this subject, while it is possible that the record of my results obtained thus far may be of immediate use to others about to enter into like investigations.

In the first place I found that the fowl-cholera organisms resemble most of the pathogenic microbes in producing acid during the earlier stages of their growth.

To determine the amount of acid quantitatively the media had to be carefully prepared. In the earlier experiments meat infusion was employed, made strictly in accordance with the directions given by Abbott in his *Principles of Bacteriology.* This broth was neutralized with sodium hydrate, phenolphthalein being employed as an indicator. That no appreciable amount of muscle sugar was present in the broth so prepared was determined by the fermentation-tube test, the bacillus coli being employed as the testing germ.* Four tubes were then taken containing 10 ccm. of the neutral broth and were sterilized in the autoclave. Of these, two were inoculated with the germ; the other two were used as controls.

The following is an example of the result of titration at the end of 24 hours' growth at 37° C. The figures given indicate the number of cubic centimetres of a decinormal solution of sodium hydrate required to neutralize the broth, phenolphthalein being here, as elsewhere, employed as the indicator:

TABLE I.

Tube inoculated with Canadian chicken cholera.	No. 1	1.00
" " " " " "	" 2	1.10
Sterile tube, not inoculated.	" 3	0.15
" " " "	" 4	0.20

It will be seen that the process of sterilization of the broth in the tubes after the broth had been neutralized, and the subsequent keeping of these tubes at 37° C., had led to a slight acid production in the broth, but the amount was very small as compared with the acid production in the inoculated tubes, in each of which the amount of acid was almost the same.

I had prepared a large quantity of the broth made from meat infusion, and used in the above series of experiments, and part of it I next employed for a fuller experiment. Taking 20 tubes I placed in each, as before, 10 cubic centimetres of the neutral broth and then sterilized.

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* Theobald Smith's ready method of making sugar-free bouillon (*Journal of Experimental Medicine*, ii (1897), 546) was published subsequent to the completion of this paper.

Three of the tubes were inoculated with a diplobacillus, sent to me by Dr. V. A. Moore and stated to have been isolated from diphtheritic lesions in fowls. Three were inoculated with the United States bacillus of fowl cholera, for which again I am indebted to Dr. Moore. Four were inoculated with the Canadian fowl cholera, four with anthrax, while five were not inoculated and were carried through as controls. In order that the amount of culture placed in each tube of the series might be the same, and might contain the same number of bacilli, I employed, not the platinum loop, but Pasteur pipettes, drawn out into fine capillary tubes. These were sterilized, and for each series one was filled with a 24-hour-old broth culture of one or other germ, and from it one drop was allowed to fall into each tube to be inoculated. Tubes so inoculated were placed in the incubator for 24 hours, at the end of which time they were titrated in the usual manner. Here again, in the table that follows, the figures given indicate the number of cubic centimetres of a decinormal solution required to neutralize the broth. It will be seen that the control tubes were in this case slightly alkaline and required an addition of acid for neutralization. Evidently the neutralization had originally been carried a little towards the alkaline side.

TABLE II.

Tubes.	1.	Uninoculated, employed as control	0.1
	2.	" " "	0.1
	3.	" " "	0.0
	4.	" " "	0.1
	5.	" " "	0.1
	6.	United States fowl diphtheria	0.3
	7.	" " " "	0.1
	8.	" " " "	0.05
	9.	United States fowl cholera	0.75
	10.	" " " "	0.4
	11.	" " " "	0.3
	12.	Canadian chicken cholera	0.5
	13.	" " "	0.5
	14.	" " "	0.6
	15.	" " "	0.6
	16.	Anthrax	0.2
	17.	"	0.15
	18.	"	0.2
	19.	"	0.2

It cannot be stated that the results of these experiments were entirely satisfactory. In two forms, namely, in the two cultures obtained from

Dr. Moore, there was a variation in the acid production, so wide, indeed, that I have been unable to account for it satisfactorily. The cultures in all cases were pure, and the only marked difference that I could make out between the various growths in these two series was that the test tubes, while containing each 10 ccm., were of unequal diameters, so that in some cases a larger and in others a smaller surface was exposed to the atmosphere. Another possible cause for this irregularity may be the fact that whereas the chicken cholera and anthrax germs had been constantly transplanted, the other two had not, having been but recently transplanted from tubes of some little age sent from Washington.

Upon endeavoring to repeat these observations and to reproduce a broth identical with that employed in this last experiment I found that the amount of neutralization, the presence of muscle sugar in minute quantities in the different broths, and still other differences rendered it almost impossible for me to hope to gain any scientific precision in my results by following the lines employed up to this time. Hence it became necessary to endeavor to settle the question of acid production by different species and by allied forms of the same species by the employment of accurately compounded synthesized media.

In pursuing this work I have tried the formulæ of most of those who have already endeavored to form satisfactory synthesized culture media, and have further studied some fifty media made up in accordance with my own ideas. The work was very interesting, though at times very discouraging. It was my aim not so much to arrive at the formula of a medium specially suitable for the fowl cholera germ, but rather one which could be substituted for ordinary broth in ordinary work. There exist several media upon which other pathogenic germs, like those of anthrax and the bacillus pyocyaneus, will grow, but singularly few upon which the bacillus of chicken cholera will flourish. This difficulty in getting the bacillus of chicken cholera to grow in these media is wholly in harmony with the observations of Pasteur, who noted that it would not grow in various vegetable infusions. The great difficulty is in the addition of those elements or salts which are required in very minute quantities, as again in the proper addition of the calcium and magnesium salts. These latter are inclined to precipitate at an inopportune moment, a most undesirable feature. Not being able therefore to gain any satisfactory synthesized media, I was, for this work on chicken cholera, finally compelled to fall back again upon the ordinary broth. Liebig's extract of beef was now used in place of meat infusion, for I have found it less liable to contain fermentable matter. Four grammes of the extract were taken, dissolved in distilled water; 1 per cent of Witte's peptone and ½ per cent of rock salt were added. These ingredients were thoroughly dissolved, neutralized to phenolphthalein with a caustic potash solution and sterilized, the tubes filled and once more sterilized. In all cases the media were tested in the fermentation tube with the bacillus coli to determine the absence of fermentable matter. All the media used so far have given a negative reaction.

With this medium the first results obtained appeared to be hopeful and to give further proof of the close relationship between the European and Canadian forms. Thus I inoculated three tubes with equal quantities of a culture of the Canadian germ and three from a culture of the European form from the laboratory of Professor Kitt in Munich and obtained the following results:

TABLE III.

1.	Uninoculated tube as check	0.2
2.	" " "	0.2
3.	Canadian chicken cholera	0.4
4.	" " "	0.4
5.	" " "	0.3
6.	European fowl cholera from Munich	0.3
7.	" " " " "	0.3
8.	" " " " "	0.4

These results seemed to indicate a very close relationship between the two germs, which, it may be added, were possessed of very nearly the same virulence.

Similar results were obtained when I employed broth of the same composition containing 1 per cent glucose and 1 per cent lactose. The method of preparation was the same as in the previous experiments, but here the determination was made at the end of 48 hours, and a viginti-normal solution of sodium hydrate was employed in place of a decinormal, the titration being thereby accomplished with much greater ease and sureness.

TABLE IV.

	Plain Broth.	1% Glucose.	1% Lactose.
Tubes as check, uninoculated	0.5	0.6	0.5
	0.3	0.8	0.6
European germ (Kitt)	0.7	2.7	1.1
	0.7	2.4	1.0
Canadian germ	0.7	2.5	1.0
	0.6	2.7	1.1

These figures again favored the contention that the two forms are very closely related, the difference between the acid production in the different media being very slight. It is interesting to note the great increase in acid production brought about by the addition of the sugars.

But a more extensive and very carefully conducted series of observations showed that no absolute reliance can be placed upon this feature of acid production, so far as regards the micro-organism of chicken cholera.

The following table shows the results obtained by determining the acid production at the end of 24 hours in tubes of the same broth as that used in the previous series of observations, these tubes being inoculated with equal amounts of cultures of fowl cholera germs that had been subjected to different influences. A viginti-normal solution of sodium hydrate was used, and the figures represent in ccm. the amount added to neutralize 10 ccm. of broth culture.

TABLE V.

	Neutral Broth.	1% Lactose.	1% Glucose.
No. 1	0.3	0.8	0.7
	0.35	0.8	0.6
	0.3	0.9	0.7
No. 2	0.2	2.0	4.5
	0.3	2.2	4.1
	0.3	2.4	5.1
No. 3	0.3	2.0	3.9
	0.3	2.1	4.3
	0.3	2.2	4.2
No. 4	0.4	1.3	1.7
	0.4	1.2	1.3
	0.4	1.2	...
No. 5	0.4	1.2	1.5
	0.4	1.1	1.9
	0.4	...	1.5
Uninoculated tubes carried as check	0.05	0.6	0.7
	0.04	0.7	0.7
	0.04	...	...

No. 1. Bacilli of European fowl cholera passed through the fowl 10 weeks previously, and cultures since renewed every third day. This germ proved fatal to a rabbit in 6 hours, 3 cc. being inoculated intravenously.

No. 2. Bacilli from Canadian outbreak, from a broth culture taken direct from one of the St. Anne's fowls and preserved in a sealed tube for about 5 months.

No. 3. Bacilli from Canadian outbreak. Broth culture made from rabbit which had been infected through the digestive tract and had succumbed in 36 hours. This culture had been kept in a sealed tube about 4½ months.

No. 4. Bacilli from Canadian outbreak passed through series of rabbits from rabbit which had been inoculated intravenously and had succumbed in 6 hours. This culture had been hermetically sealed for 4 months.

No. 5. Bacilli from Canadian outbreak passed through a turkey. Culture from liver renewed every third day for 3 months and 9 days.

It is to be observed from this table that there is little variation between the different series in neutral broth, but this is far from being the case in those containing sugar. A difference of 0.1 ccm. of a vigintinormal solution of the alkali added to 10 ccm. of the culture—that is, of 1 per cent in the amount of solution added—is inconsiderable, and in determinations of this nature, even when made with the greatest care, cannot be regarded as indicating any profound differences in acid production. But if this table be compared with Table 3 it will be seen that forms here giving very closely allied results to those with plain broth, namely, the European and Canadian forms, gave in other determinations wider variations, while with sugar-containing broths they gave results which were closely parallel. It is unsafe, therefore, to depend upon the apparently satisfactory results shown in this one table concerning the acid production by allied forms in neutral sugarless broth.

When we compare the results gained from lactose and glucose broths we find still greater discrepancies. So marked are they that it is immediately evident that the variations in virulence of at least one member of the group of the bacilli of hæmorrhagic septicæmia are accompanied by equally wide variations in acid production, and so far as this table may be depended upon it would seem to a certain extent that these variations are inverse, for the least virulent forms (Nos. 2 and 3) produced most acid in both lactose and glucose broths.

It is quite probable that more stable forms of bacteria may give more constant results; but it is with these unstable bacteria that we have the greatest difficulties, and are most often led to question whether we are dealing with distinct species, or merely with varieties of a common form, and so far as these observations extend it would seem evident that little or no dependence can be placed upon determinations of comparative acid or alkali production as helping to solve the problem of relationships.

CONCLUSIONS.

(*a*). The bacillus isolated by me from the outbreak of choleraic diarrhœa corresponds closely in all essential particulars with that of European chicken cholera.

(*b*). It differs, consequently, from that isolated from previous outbreaks of choleraic diarrhœa in the United States.

(*c*). A study of the acid production by this and allied bacilli would seem to show that the amount of acid produced by so variable a microbe varies greatly and is incapable of affording a further means of distinguishing between allied forms.

ADDENDUM.

ON SUNDRY SYNTHESIZED MEDIA.

In pursuing the subject of synthesized media the paper of J. J. McKenzie,* of Toronto, was taken as a working basis. The various media as given there were tried with very little success; from these others were composed, the best of which will be found below together with the results obtained. In all there were about fifty formulæ tried. including five or six taken from McKenzie's paper. In all cases the media were neutralized, phenolphthalein being used as indicator.

The first of this series was very simple, as follows:

Potassium phosphate	0.15
Ammonium chloride	0.80
Glycerine	5.00
Water	100.00

Six tubes were prepared and were inoculated with diphtheria, European fowl cholera, Canadian fowl cholera, pyocyaneus, Asiatic cholera, and the germ of the Pictou cattle disease. At the end of 24 hours the tubes inoculated with the germs of European fowl cholera and Asiatic cholera showed only slight growths, while the others showed nothing. At the end of 72 hours pyocyaneus appeared. None of the growths at this time were very vigorous. This same formula with the addition of 0.1 gramme of potassium sulphate showed in addition to the above at the end of 72 hours a growth in the tube inoculated with the germ of the Pictou cattle disease. The growths in this mixture were not vigorous. The next medium which proved itself worthy of passing notice was as follows:

Potassium phosphate	0.4
Potassium nitrate	0.8
Asparagin	0.8
Lactose	0.5
Glucose	0.5
Water	200.0

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* *Journal of the American Public Health Association*, October, 1895.

This was inoculated with the same germs as the previous tubes, save that a putrefactive form was substituted for the Asiatic cholera. In 15 hours all show a slight growth, while that of pyocyaneus is quite vigorous. Diphtheria seems to grow fairly well after the first day and increases considerably in cloudiness.

Another medium which proved to be superior to the foregoing was as follows:

Ammonium succinate	0.8
Potassium phosphate	0.4
Potassium sulphate	0.2
Ammonium chloride	0.3
Asparagin	0.5
Lactose	0.5
Glucose	0.5
Water	200.0

Six tubes were taken and inoculated with the bacillus violaceus, pyocyaneus, coli, the germ of the Pictou cattle disease, European fowl cholera and that from the Canadian outbreak. At the end of 24 hours there is observed to be a very profuse growth in the case of the first three, being equal in all to that obtained ordinarily in broth, while that of bacillus coli is much better than the best growth obtainable in the same time in broth. The other three tubes remained sterile. After 48 hours pyocyaneus brought forth its characteristic color.

A still better formula was as follows:

Ammonium phosphate	0.5
Potassium nitrate	0.5
Potassium chloride	0.3
Ammonium sulphate	0.2
Asparagin	0.5
Lactose	0.5
Glucose	0.5
Water	200.0

This was inoculated with the same germs as the preceding, with a similar result excepting that violaceus was much more vigorous than in the preceding. Agar plates were made showing a good growth of the three forms above mentioned, as also of bacillus mesentericus, but there was not the character to the colonies such as is seen where broth is used.

I will mention but one more which I think is the best tried. The formula is as follows:

Ammonium phosphate	0.5
Potassium nitrate	0.5
Potassium chloride	0.3
Ammonium sulphate	0.2
Ammonium succinate	0.5
Lactose	0.5
Glucose	0.5
Water	200.0

This is practically the same as the one above save that instead of asparagin, ammonium succinate is used. Here bacillus violaceus, pyocyaneus and bacillus coli grew well. This medium was made up also with 7 per cent gelatine and gave similar results in examining water as did gelatine prepared with ordinary broth.

The following results were obtained with an agar preparation of this medium as compared with ordinary broth agar:

	Number of Colonies.	
	Broth.	Synthesized medium.
1.0 cc. water, 48 hours,	70	2
1.0 cc. water, 48 hours,	81	6
0.1 cc. water, 48 hours,	14	0
0.1 cc. water, 48 hours,	11	0
Pyocyaneus	1248	18,720
B. coli	156	260
Violaceus	No growth on either.	

The colonies were large, very distinct, and the edges were well defined. On the whole the colonies which appear are much larger than those seen on broth plates of the same age. There seems to be in this medium some element which is in such a form that the bacillus pyocyaneus has a special affinity for it and which seems to greatly increase its growth.

From the work which I have done in this line it does not seem probable that a generally serviceable medium of simple formula can be prepared, as many of the elements are required in very minute quantities. Magnesium and calcium are needed to insure success, but the great difficulty, as previous workers on this subject have noted, is to get them in such a form that they will not precipitate when subjected to the heat of the sterilizer.

DIFFUSE SARCOMA OF THE CEREBRAL AND SPINAL PIA MATER.

By GEORGE H. WEAVER, M. D.

(From the Pathological Laboratory of Rush Medical College.)

Tumors of the soft membranes of the brain and spinal cord, especially those of a diffuse character, are comparatively rare, and a careful study of each individual example is of distinct value. The following case was kindly offered me for study by Prof. Ludvig Hektoen, to whom I am indebted for much assistance in the preparation of my report.

Primary tumor of cerebrum, secondary diffuse sarcoma of the cerebral and spinal pia mater.—W. C., male, aged 11 years, entered the Presbyterian Hospital December 31, 1896, in the service of Dr. A. C. Cotton. Clinical diagnosis, tubercular meningitis.

Family history negative. No tubercular or specific history obtainable. Present illness began September, 1896, with convulsions, in which the whole body was rigid and the eyes fixed. Before the convulsions the patient was dizzy and vomited; after them he would sleep for a time, and upon waking would be delirious. At times he was confined to bed.

Examination on admission: Confined to bed, poorly nourished, skin warm and moist, countenance staring and flushed at times, pulse regular and rapid, Cheyne-Stokes respiration, bowels constipated, pain in head and neck; only partly conscious, moves but little, but there is no paralysis; eyes bulging, pupils widely dilated, sight much impaired, the right eye insensible to light, the left to sensation; nystagmus marked, double optic neuritis; temperature 98.8° F., pulse 100. Hyperæsthesia over entire body. Pain on percussion over the cranium. *Taches cérébrales.* From this period until death there were recurring convulsive paroxysms, sometimes several in a day, and again only at intervals of several days. There were, later, delirium and involuntary evacuations. Death took place April 13, 1897.

Autopsy by Dr. Hektoen.—Body small and extremely emaciated; head large; thyroid gland large. Larynx and trachea normal. Pleuræ smooth and empty. Over the anterior surface of the right lower lobe is a circumscribed area in which the pleura contains numerous grayish-yellow, pinhead and larger, disc-like nodules. The peribronchial lymphatic glands contain tubercular areas. Lungs normal save for circumscribed areas of grayish-red consolidation in the lower lobes. Heart small; endo- and myocardium normal; pericardium smooth. Aorta normal. Spleen, kidneys, pancreas, liver, stomach, intestine and adrenals normal. Mucous membrane of urinary bladder red, rough and covered with shreddy material.

Upon opening the cranial cavity a large amount of very slightly turbid fluid runs out. Dura mater normal. The pia mater upon the optic commissure, the medulla and the upper part of the spinal cord is the seat of a reddish, indistinctly nodular thickening. Around the apex of the left frontal lobe is a similar area. The entire posterior surface of the spinal cord is the seat of marked thickening, so that the pia is reddish and somewhat lobulated, and the volume of the cord appears to be greater than normal. It fills the dura, and when the latter is cut masses are forced out through the opening. The ventricles of the brain are greatly dilated, containing a clear fluid.

Anatomical diagnosis.—Sarcoma of pia mater of brain and spinal cord. Hydrocephalus. Broncho-pneumonia. Acute cystitis. Chronic tuberculosis of the peribronchial lymph glands and pleuræ.

Description of the brain.—There are irregular areas of a gelatinous, opaque, whitish-gray substance in the pia mater, scattered over the anterior surface of the medulla and the pons and spreading out over the lower surface of the lobes of the cerebellum. The largest area upon the cerebellum is on the right lateral hemisphere, being 4.5 cm. from before backward and 2 cm. wide at the thickest part. This lies parallel to the fissure between the lateral and median lobes, extending outward from the fissure. This area is not elevated above the surface but appears to involve the underlying substance. From this area a thinner growth extends continuously over the lower surface of the median lobe, becoming less dense as it approaches the left lobe. Upon the left lobe, lying in a position symmetrical to that on the right, is an area of growth 1.5 cm. antero-posteriorly and 0.75 cm. transversely. This is slightly elevated above the surface and somewhat firmer than the corresponding area on the opposite side. Scattered over the under surface of both lateral cerebellar lobes and along the front of the medulla and pons are areas of growth varying in size from those which are very minute to those 0.5 cm. in diameter. The largest of these are elevated above the surface and are firmer than the larger areas upon the cerebellum. They appear to bear no relation to the vessels in their distribution. On the upper surface of the optic commissure and optic nerves at their origin are globular masses of growth 0.5 cm. in diameter, which are partly continuous, thus forming an irregular, uninterrupted mass along the optic commissure and nerves. The apex of the left cerebral hemisphere is soft and somewhat collapsed. The surface for about 3.5 cm. from the apex, on all sides except below, presents the same sort of growth as that found on the cerebellum. It is, however, of a more pinkish-gray color. The tissue underlying this surface growth is very soft. A few small discrete masses of growth are seen at short distances from the larger area over the surface of the left cerebral hemisphere.

The brain was hardened in Müller's fluid containing 5 per cent of formalin. On cutting the left cerebral hemisphere into vertical sections, each 2 cm. thick, it is found that the centrum ovale of the left apex is replaced by a tumor extending antero-posteriorly 5 cm.; its diameter at a depth of 2 cm. is 5 cm., and at a depth of 4 cm. is 4 cm. The growth is extremely soft, and of a pinkish-gray color. In the sections the tumor mass tends to fall away from the surrounding tissue. The lateral ventricle is empty, very much dilated, its wall smooth, and the choroid plexus unaltered. Corresponding sections of the right hemisphere show the lateral ventricle to be similar to the left. This hemisphere is otherwise normal.

The fourth ventricle is filled to distension with a reddish and in parts grayish tumor mass. This is very soft, contains much blood and is continuous with the walls of the ventricle. It measures antero-posteriorly 4.5 cm., transversely 3.5 cm., and vertically 2.5 cm.

The spinal cord.—The cord, which was hardened in 5 per cent formalin, appears very much enlarged in the dorsal and lumbar regions. In the lower dorsal region it measures 1.75 cm. antero-posteriorly and 2.5 cm. transversely. The growth externally is slightly nodular and uneven, opaque and whitish-gray. In the upper dorsal region the blood-vessels are large and prominent. The growth extends over and between the nerves of the cauda equina. On section the cord itself is found to be of about normal size, the apparent enlargement being due to a growth of new tissue about it. The tumor growth is most abundant posteriorly, and is thickest in the lower dorsal and lumbar regions, where it is 0.75 cm. in thickness; it becomes much thinner in the cervical and upper dorsal regions. The growth in front of the cord also extends throughout the entire length, being more largely developed in the lower dorsal and lumbar regions. It is nowhere more than 1 or 2 mm. thick. Laterally the growth is more abundant in the lower portion of the cord, where it is from 2 to 3 mm. thick and completely surrounds the posterior roots and to a less extent the anterior roots of the spinal nerves. The posterior roots appear very large from the tumor deposit. In the upper part of the cord the lateral growth exists as a very delicate, thin layer.

MICROSCOPIC EXAMINATION.

Spinal cord.—Weigert's stain. The cord is usually normal except in some places along the posterior border, where it is invaded for a short distance by the tumor.

Hæmatoxylin and eosin. The tumor is usually sharply limited at the border of the cord by a fibrous structure consisting of the altered pia mater. There is a growth of tumor tissue along the normal trabeculæ which pass from the pia mater into the cord. Posteriorly the cord is directly invaded by the tumor for a short distance. The tumor is everywhere of the same structure, consisting of closely-packed, small, round cells, with deeply-staining nuclei. The intercellular structure is usually but little developed, and in most places appears as a homogeneous material, staining faintly with eosin. Where the cells are not so closely packed it is finely fibrillated. In some places the connective tissue framework is more largely developed, appearing as quite heavy bands, but with little tendency to any regularity of arrangement. The blood-vessels are large and numerous with well-developed walls. In the cervical and upper dorsal regions the tumor is less than in the lower parts, but exists upon all sides. It is here thinnest at the sides and most marked posteriorly. The nerve-roots are here more or less surrounded by the growth, which has not penetrated the nerves themselves. In the lower dorsal and lumbar regions the growth is also least laterally and most developed posteriorly. The posterior nerve-roots, and to a less degree the anterior roots, exhibit an infiltration of the epineurium and perineurium, but the endoneurium has not been invaded by the tumor. Some of the larger vessels and bands of fibrous tissue have undergone hyaline degeneration. In the cauda equina the growth surrounds the nerves, but only in a few places was the endoneurium penetrated by tumor cells.

Brain.—The tumor in the apex of the frontal lobe consists of small round cells with deeply-staining nuclei, like those in the spinal growth. The arrangement of the vessels and the trabeculæ of the fibrous framework produces a somewhat alveolar appearance in places. The growth in the cerebral pia mater is similar to that in the spinal.

Sections were made of the tumor growth in the fourth ventricle, including the adjacent cerebellar tissue with its covering of pia mater, which appeared very slightly thickened. There is everywhere a thin layer of new growth following the pia mater over the convolutions of the cerebellum. The cells penetrate to a very slight extent the brain substance. The blood-vessels are very numerous, many being of large size. There are large and small areas of hæmorrhage into the tumor, some being quite recent with well-preserved blood corpuscles; others old, with remnants of blood-cells, fibrin and blood pigment.

Lungs.—In the lungs, on and near the pleura, are small areas of tubercular infiltration, containing foci of caseation, giant cells and tubercle bacilli. In the lungs are some areas of cell accumulation which resemble those in the tumor of the brain and cord. In these no caseation or giant cells are seen. From the histology of these areas it is impossible to decide whether they are metastatic tumors or not.

I have collected from the literature ten reports of diffuse meningeal sarcoma, of which the following are abstracts:

I. (Coupland and Pasteur.*) Female, aged 22 years. Course of illness about 4 months. Began with headache and morning sickness; later there were pains in the right shoulder and arm. A month before death pain along the spine; two days before death constant occipital pain radiating to the right shoulder and arm, double optic neuritis, paralysis of both external recti, tenderness of scalp, hyperæsthesia along the spine, weakening but not paralysis of the legs, and absence of knee-jerk; sphincters acted normally; no convulsions.

Autopsy.—Soft, jelly-like masses of new growth scattered over the meninges beneath the dura in the posterior fossa, about the body of the sphenoid and backward almost continuously to the foramen magnum. Medulla and pons uniformly invested with similar growth in which both sixth nerves were completely embedded. Spinal cord uniformly ensheathed in a semi-translucent, softish growth of a grayish-white color, the tumor being most developed on the posterior and lateral aspects of cord. Nerves of cauda equina surrounded by thin sheaths of new growth, which at intervals expanded into small tumors. The cord itself normal in shape and appearance throughout. The new growth was a small round-celled sarcoma, which followed the trabecular ingrowths of the pia into the cord. Brain normal.

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* *Trans. Path. Soc.*, London, xxxviii (1887), 26.

II. (Coupland and Pasteur.*) Female, aged 4½ years. Duration of illness about 3 months. Following a fall the child became irritable, developed a squint, and at the end of a month was unable to walk. Pain in upper part of back and head was followed by sudden blindness six weeks from the onset. Two months from the beginning was conscious and intelligent; pupils dilated and insensitive, blindness, pain in back and head. Optic discs gray and indistinct. General muscular weakness without atrophy, normal tactile sense, *taches cérébrales*. No convulsions.

Autopsy.—A layer of grayish material covered the under surface of the cerebellum over the middle and part of the lateral lobes, varying in thickness and extending for about half an inch into the nervous tissue. Middle lobe almost entirely replaced by a soft, gray, vascular growth. Pia-arachnoid over medulla and pons normal. Thin layer of new growth on left side of optic chiasm and nerve, with similar patch, about the size of a pea, on under surface of left lower temporal convolution. Lateral ventricles greatly distended. Basal ganglia normal. Spinal cord appeared enlarged at upper part with small scattered hæmorrhages in the gray matter. Posterior aspect of the cord covered throughout by new growth ¼ to ⅛ inch thick in lumbar region. The tumor was a small round-celled sarcoma.

III. (Ollivier.†) Male, aged 11 years. Duration of disease about 6 months. There were paroxysmal headaches, retraction of head, convulsions, and later vomiting. Two days before death blindness with dilated pupils and pains in the limbs. The day before death acute pains throughout the body, but worst in the back; sensation and motion unimpaired. Patient conscious. Convulsions, constipation and nausea; no vomiting.

Autopsy.—There was a uniform pinkish formation extending over the posterior surface of the cord, averaging one line in thickness, thinner toward lateral aspects of the cord and blended with arachnoid. It was fairly firm, like unsoftened cerebral tissue, and traversed by small blood-vessels. There was a tumor in the cerebellum.

IV. (Hadden.‡) Although described as chronic cerebro-spinal meningitis, this case was evidently one of diffuse meningeal sarcoma. Male, aged 13 years. Duration 14 to 17 months. An injury to the head 17 months before death was followed by pain in the head and vomiting. 14 months before death sickness in the morning, pain in the head, fainting and attacks of dizziness. Two or three months before death weakness of legs, loss of control over bladder, convulsions, followed by stupor, occurring as frequently as 4 or 5 times a day and ceasing a few weeks before the fatal ending. The patient was drowsy and nearly blind, the pupils being equal, dilated and sluggish. Temperature usually normal or sub-normal, being only twice above 100° F.

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* *Trans. Path. Soc.*, London, xxxviii (1887), 26.

† Traité des maladies de la moelle épinière, t. ii, p. 490, obs. cxxxviii, Paris, 1837.

‡ *Trans. Path. Soc.*, London, xxxvi (1885), 13.

Autopsy.—Under surface of cerebellum almost entirely coated with an opaque white material varying in thickness from 1 to 4 mm. Upper surface of cerebellum and adjacent parts of the cerebrum similarly but less extensively affected. Tips of temporosphenoidal lobes showed the same condition in a minor degree. Much thickening and white opacity about optic tracts and chiasm. Lateral ventricles distended with clear fluid. No tumor in the brain. Beneath arachnoid on posterior surface of lower dorsal and lumbar cord a thick, white layer similar to that upon cerebellum, which presented numerous bleb-like elevations. Cervical and upper dorsal cord free.

The white thickening over cerebellum and cord consisted microscopically of round and spindle-shaped cells with a considerable amount of new connective tissue in interlacing bundles. The vessels were large. No tubercles.

V. (E. L. Fox.*) Male, aged 29 years. Duration of disease not given (patient came to hospital after being sick for three months, and probably did not live long afterward); began with shooting pains in arms. Gradual flexion of fingers developed. Difficulty in urinating and priapism. On admission anæsthesia of legs, thighs, abdomen and chest to third rib, and paralysis of lower extremities.

Autopsy.—The cord seemed generally larger than normal with a marked swelling posteriorly at its upper portion midway between cervical bulb and second cervical vertebra. On section this was found to be a tumor, 1½ in. long and ¾ in. broad, with well-defined margins of a purplish color and tolerably firm. Cord smaller than normal, the great apparent increase in size being due to a layer of yellowish-gray substance fully ¼ in. thick, enveloping cord on all sides. This growth extended from highest portion of cord to cauda equina. In pia mater covering pons and medulla much thickening and rounded masses of exudate extending along the vessels as far as fissure of Sylvius.

Microscopically the tumor was a spindle-celled sarcoma.

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* *Bristol Medico-Chirurg. Journ.*, i (1883), 104.

VI. (G. H. Bennett.*) Male, aged 48 years. Duration about 11½ months. Began with weakness in legs. No headache or other cerebral symptoms except dizziness. Vision good. Dull aching in back and lower limbs. For six weeks before death patient could not stand; suffered from constant severe pains in legs and from attacks of uncontrollable vomiting.

Autopsy.—Areas of softening in cerebrum and cerebellum. Pia mater of cord thickened, especially its posterior aspect, most marked below and gradually diminishing upward. Posteriorly, membrane studded throughout with round and oval, isolated and confluent, white nodules, varying in size from mustard seed to pea. These growths surrounded posterior roots. None visible to naked eye in front or laterally. The growth was a round-celled sarcoma.

VII. (Westphal.†) Female, aged 15 years. Duration a little over a month. At first headache, dizziness and pain in back, and two weeks later convulsions. Mind dull, pupils irregular, slight paresis, pain over spine on percussion, no choked disk. Before death severe headache, dizziness, vomiting, paralysis of ocular muscles and absence of knee-jerk. No fever. Death took place in a convulsion.

Autopsy.—Grayish-white and in parts more reddish thickening of pia-arachnoid, extending from optic chiasm backward over pons and medulla and outward somewhat over temporal lobes. Cranial nerves more or less imbedded in tumor thickening. Lateral ventricles contained blood-tinged fluid. In and near left optic thalamus three firm, smooth tumors of a red and grayish-white color, one being connected with tela choroidea. Pia-arachnoid of cord, especially posteriorly, presented grayish-red tumor growths, slight above, but increasing downward, becoming in dorsal region a prominent covering. Cord itself normal. Metastatic nodules in lung.

The tumor was a richly nuclear, round-celled sarcoma, with numerous vessels scattered between the cells. The cord was not invaded. The spinal nerve-roots were imbedded in but not invaded by the growth. The tumor had implicated the cranial nerves.

VIII. (Virchow.‡) Male, aged 30, copper-worker; attacks of colic, paresis and toward the last almost total blindness.

Autopsy.—On surface of hemisphere single yellowish-brown pigment spots. At base of brain a more diffuse, confluent deposit in the soft membranes of a melanotic material, extending laterally and thickest between chiasm and pons. At apex of middle lobe and on sides and middle of cerebellum growths as much as 3 lines in thickness. Diffuse growth along various cranial nerves, optic and olfactory being imbedded in it. The growth extended into the nerves and completely infiltrated the Gasserian ganglion, but did not extend beyond the foramina. Eyes normal. On posterior surface of cord in upper part isolated, flat, almost black tumors; below in dorsal and lumbar regions these became continuous, the cord being covered by a layer of growth 2 to 3 lines thick. In cervical portion, posteriorly and laterally and in front throughout, only blackish-brown pigmentation was apparent. Nerves of cauda equina and most of other spinal nerves covered by growth forming in places nodules. The tumor was a round and spindle-celled melanotic sarcoma. In a few places pigment was absent.

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* *Trans. Clin. Soc.*, London, xviii (1885), 168.

† *Arch. f. Psychiatrie*, xxvi (1894), 770.

‡ Virchow's *Archiv*, xvi (1859), 180.

IX. (G. Lentz.*) Male, aged 23 years. Duration about 12 months. Began with pain in back, later in arms and legs; weakness of muscles, first in legs, later in arms and trunk. At end of 6 months pain and tenderness on percussion over spine and exaggerated patellar reflexes. Optic neuritis with normal pupillary reaction. One month before death patient was bed-ridden, mind dull, pupils irregular and sluggish, bilateral choked disk, marked paresis and atrophy of muscles. Patellar reflex present on right side only. Sensibility reduced in legs, arms and parts of back. Later vomiting and cystitis.

Autopsy.—There was a tumor involving corpus callosum and laterally invading the hemisphere. Lateral ventricles distended with fluid. A cavity in upper part of cord. Whole posterior extent of cord covered by a pale gray mass about 1 mm. thick. In dorsal region growth extended to the sides of cord. From lumbar region downward whole cord encased in a gray, homogeneous, jelly-like mass 0.5 cm. thick. Cord was of normal appearance only in the lower portion.

The mass of the tumor consisted of closely-packed, short, oval or roundish cells, separated by scanty interstitial tissue, in places distinctly fibrillated. There were abundant vessels with well-developed walls, some having undergone hyaline changes. The pia was usually sharply differentiated from the cord, and in places the tumor penetrated along the pial septa. Here and there the connective tissue was arranged so as to form a meshwork in the spaces of which the cells were accumulated. Large cells of endothelial type were found singly and at times in groups. Limited portions of cord were directly invaded by the tumor. The cerebral tumor was of similar structure and hæmorrhagic.

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* Ziegler's *Beiträge*, xix (1896), 663.

X. (R. Schulz.*) Female, aged 16 years. Duration about 3 months. Severe pain in back, numbness and formication in hands and arms, with increasing weakness in arms, which were paralyzed shortly before death. Patient conscious up to the last. Pain in head, nutrition good, inability to stand, hands red and swollen, tenderness on pressure over main nerves of trunk and upper limbs. No affection of cerebral nerves. Marked tenderness over whole spine, retention of urine, constipation; anæsthesia of both upper limbs and absence of the patellar reflex on right side. Clinical diagnosis was acute meningo-myelitis.

Autopsy.—Cranium and its contents normal. On opening spinal dura cord bulged, presenting a sausage-like appearance. Sub-arachnoid space occupied throughout by grayish-red mass of growth, covered by delicate arachnoid, nodular and lobulated, partly firm, in places soft and studded with hæmorrhages. In lumbar and lower dorsal regions growth completely ensheathed cord, while in the upper part it covered only posterior surface. At one point it penetrated the cord.

Microscopically growth was made up of large endothelial cells, traversed by numerous capillaries which gave it an alveolar character. It is described as an alveolar sarcoma.

Of the 11 cases, including the one here reported, in which there was a diffuse sarcomatous growth in the pia mater of the cord and base of the brain, in 6 there was no tumor of the nervous structures which could be considered primary (Coupland and Pasteur, Schulz, Hadden, Fox, Virchow). In the other five there was tumor growth in either the cerebrum, cerebellum, pons or basal ganglia. In all of these cases the macroscopic characteristics were very similar, as was also the distribution. In the cranial pia mater the growth was most abundant about the optic commissure and over the cerebellum, and spread here and there to the surface of the lower portions of the cerebrum. It usually extended more or less along the cranial nerves. The spinal growth was most extensive along the posterior surface of the lower part of the cord. The posterior growth was comparatively slight in the upper part, as was also that in front and to the sides of the cord throughout the entire length. The tumor tissue was yellowish or grayish-white, with more or less of a pink or red color in some cases. The consistence varied, but was usually rather soft.

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* *Archiv f. Psych.*, xvi (1885), 592. (Abs. in *Trans. Path. Soc.*, London, xxxviii (1887), 32.)

The microscopic characters of the tumors from the various cases have differed somewhat. Virchow's case is the only one of a melanotic growth. Schulz reports his case as one of alveolar sarcoma. In Hadden's case the tumor is said to consist of round and spindle cells, and Fox's was one of spindle-cell growth. In the two cases of Coupland and Pasteur, in those of Bennett, Lentz and Westphal, and in my case, the tumor was a small, round-celled sarcoma. In some of the cases the blood-vessels are spoken of as being large and abundant. There was usually little or no tendency for the growth to invade contiguous structures. The cord was rarely involved, and then only to a slight degree; the brain was more apt to suffer. The growth, however, showed a special tendency to infiltrate the pia mater even to its finest processes and prolongations. The cranial nerves were occasionally invaded by the tumor cells, but the spinal nerve-roots, even when imbedded in a mass of tumor tissue, were only occasionally directly attacked.

The variety of diagnoses made *intra vitam* in the reported cases indicates the divergent character of the clinical manifestations. This is largely due to the wide diffusion of the lesions and the association in many of the cases of symptoms produced by one or more tumor nodules in the brain. In my case the large growths in the cerebrum and fourth ventricle had not given rise to symptoms, and it was thought by the clinicians who had studied the case that localized tumor could be excluded from the diagnosis.

The ages of the patients varied from 4½ to 48 years. Of those in which the age is recorded, 6 were under 20 years and only one above 30 years. The course was usually tolerably rapid, the fatal termination being reached within a few months. Pain in the head and back was an early symptom. There were often pain and hyperæsthesia in the extremities, followed by muscular weakness, and later paralysis. Constipation was sometimes followed by incontinence of fæces and urine. Paralysis of the ocular muscles, partial or complete loss of sight and optic neuritis were noted. The temperature was not usually above the normal. In the early stages consciousness was often unimpaired, but later was sometimes much disturbed.

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